Investigating the standard method used by Umgeni Darvill Waste Water Works for the determination of pseudo total metal concentrations in their sewage sludge

Submitted in fulfillment of the academic requirements for the degree of

Master of Science

In the College of Agriculture, Engineering & Science

School of Chemistry and Physics

At the

Pietermaritzburg Campus

by

Nhlakanipho Seveon Ngubane

2016

ABSTRACT

Umgeni Darvill Waste Water Works (UDWWW) makes use of the "ISO 11466: 1995 soil quality extraction of trace elements soluble in aqua regia" method for the analysis of their sewage sludge. The sewage sludge produced at UDWWW has a high concentration of heavy metals. The sludge is irrigated on land as a suspension and the concentrations (with appropriate levels of uncertainty) of the heavy metal pollutants have to be determined in order to allow for proper classification of the sewage sludge. The aim of this study was to use the cause and effect diagram to identify the main sources of uncertainty in the determination of metal concentrations in the sewage sludge digested with the ISO 11466 method. The metal pollutants investigated were As, Cd, Cr, Pb & Hg. The sources of uncertainty came from the set of operating procedures including the sample preparation techniques and the detection systems used; Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES); Graphite Furnace Atomic Absorption Spectroscopy (GFAAS); Vapour Generating Accessory with Atomic Absorption module (VGA-77 AA) and/or ICP-OES module (VGA-77 ICP-OES). The four main sources of uncertainty were the sampling strategies, instrument (repeatability of the detection system), acid digestion & instrument calibration of which the last two were found to have small and negligible contributions. For all detection systems used, the precision in the concentrations of the metal pollutants was measured as the %RSD and then used to calculate the overall uncertainties. The precision for the analysis of all metal pollutants in the ICP-OES was high as indicated by low %RSD. All of the concentrations obtained with all the detection systems used led into classifying the sewage sludge as best quality according to the South African Sludge Management Guidelines. The accuracy of these analyses was not determined due to the lack of certified reference materials. The random subsampling of sieved and ground sewage sludge was shown to be effective and pivotal in reducing errors thereby increasing the precision of the concentrations for all the metals analysed. The GFAAS was used as an alternative detection system for the analysis of the cadmium due to the interference observed in the ICP-OES. The precision of the GFAAS was lower compared to the ICP-OES but the concentration determined was found to be more accurate and more realistic. The mercury content was analysed using the VGA-77 with AA module as well as with the ICP-OES module. Both detection systems measured the mercury levels with a high precision but a higher accuracy obtained using the VGA-77 ICP-OES qualified this detection system to be preferred over the VGA-77 AA. Comparisons of all the metal pollutant levels in the sewage sludge with the data available in literature showed that the analyses performed was reasonably correct. The precision measured for all the detection systems used was found to be better than that predicted using the Horwitz equation and this was indicative of a good sample homogeneity.

DECLARATION – PLAGIARISM

I, Nhlakanipho Seveon Ngubane hereby declare and certify that:

- The research reported in this dissertation, except where otherwise indicated and properly acknowledged, is my original work conducted independently by me at the School of Chemistry and Physics in the University of KwaZulu-Natal Pietermaritzburg Campus.
- This dissertation has not been submitted for examination for any degree at any other university.
- This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from the other person.
- This dissertation does not contain other persons writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    - Their words have been re-written but the general information attributed to them has been referenced.
    - Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
- This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the reference section.

Signed: .....

Nhlakanipho Seveon Ngubane

I as the candidate's supervisor have/have not approved this dissertation for submission

Signed: .....

Dr Andrew Kindness

ACKNOWLEDGEMENTS

I would like to thank the School of Chemistry and Physics at the College of Agriculture, Engineering & Science for allowing me to conduct this research at their facilities. This work began in 2012 under the supervision of Dr. Colin Southway and was meant to be completed in 2013 of which did not come to fruition due to several circumstances. I thank him nevertheless for believing I had what it took to do this research. To the Technical staff at the School of Chemistry, Mr Bheki Dlamini & Bab' Robert who assisted me relentlessly with setting up the laboratory experiments, I am very grateful.

To Prof. Ross Robinson, thank you for taking prior action when I reported the problems I was experiencing with getting my work supervised and guided into a sensible thesis. Your compassion towards students is one of the many reasons we love and wish nothing but the best for you. To my family and friends, I know you've been asking a lot about when am I finishing this and there were times where I was not certain if I will ever get this done but through your support and prayers it all worked out well at the end. To my current employer, AECI's Experse Surfactant Technologies, thank you from the bottom of my heart for allowing me some time off & the resources needed for me to complete the write up of this thesis.

Last but definitely not least, to Dr. Andrew Kindness from the University of Aberdeen in Scotland, you are the reason for all of this work. You came at a point where I was ready to give up. Offering me guidance from the other side of the world through emails is the reason we were able to get this thesis reshaped into a form that made sense and ready for submission. You have taught me so many things I didn't know about doing a scientific write up and how to look critically at the results one obtains from experiments. I can write paragraphs about my gratitude to you. You have been a true Godsend. For these reasons and many, I dedicate this thesis to you.

Table of Contents

ABSTRACT i

DECLARATION – PLAGIARISM ii

ACKNOWLEDGEMENTS iii

LIST OF FIGURES ix

LIST OF TABLES xi

LIST OF EQUATIONS xiv

CHAPTER 1 1

1. Introduction 1

1.1 Sewage sludge 1

1.1.1 Generation of sewage sludge and its various forms 1

1.1.2 Disposal of sewage sludge 2

1.1.3 Benefits of sewage sludge 2

1.1.4 Concerns of sewage sludge 3

1.1.5 Composition of sewage sludge 5

1.2 Sampling 6

1.2.1 Description of sampling and its importance 6

1.2.2 Sampling Techniques 7

1.2.2.1 Non-probability random sampling 7

1.2.2.2 Stratified random sampling 8

1.2.2.3 Cluster sampling 8

1.2.3 The preservation of samples 9

1.3 Estimation of sampling errors and the effect of particle size 10

1.3.1 Errors in analysis 10

1.3.2 Particle size analysis 11

1.3.2.1 Sieve analysis 12

1.3.3 Measurement uncertainty & Uncertainty budget 12
1.3.3.1 The process of measurement uncertainty estimation 13
1.3.3.2 The importance & use of Fishbone diagrams 15
1.4 Parameters and tests for method validation 16
1.4.1 Precision, Accuracy, Bias and Recovery 16
1.4.2 The working range & linearity 17
1.4.3 Robustness & Selectivity 18
1.4.4 Limit of Blank, Limit of Detection & Limit of Quantitation 18
1.4.5 Reference Materials 20
1.5 Sample preparation 21
1.5.1 Preparation of solid samples for inorganic & organic analysis 21
1.5.1.1 Decomposition Techniques 21
1.5.1.2 Acid Digestion 21
1.5.1.3 The chemistry of Acid Digestion 23
1.5.1.4 Microwave-assisted wet digestion 23
1.5.1.5 Dry ashing 24
1.6 Instrumentation 25
1.6.1 Background 25
1.6.2 Atomic spectrometry 25
1.6.3 Flame Atomic Absorption Spectroscopy (FLAAS) 26
1.6.4 Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) 27
1.6.6 Inductively Coupled Plasma Mass Spectroscopy (ICP – MS) 27
1.6.5 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) 30
1.6.6 Vapour Generation Techniques 31
1.7 Using standardized procedures for quality control and improving accuracy 32
1.7.1 Quality assurance and Standard Operating Procedures 32

1.7.2 Internal Quality Control 32
1.7.3 External Quality Control 32
1.8 Aims of the project 34
CHAPTER 2 35
2. Optimization & Methodology 35
2.1 Determination of moisture and solids content 35
2.2 Sampling Techniques 35
2.2.1 Digestion of sieved and ground sewage sludge 35
2.3 The acid digestion method (ISO 11466) 36
2.4 Optimization and method development for the ICP-OES 38
2.4.1 The ICP-OES used for this research 38
2.4.2 The operating conditions and specifications of the VARIAN 720-ES ICP-OES 40
2.4.3 Selection of the appropriate wavelengths for each of the elements 41
2.4.4 The calibration curves for the analytes 44
2.5 The principles, optimization and method development for the GFAAS 54
2.5.1 The principles of the GFAAS 54
2.5.2 The GFAAS used for the current research 55
2.5.3 Operating conditions and specifications of the THGA graphite furnace 56
2.5.4 Calibrating the graphite furnace 58
2.5.5 Optimizing the graphite furnace 59
2.5.6 Calibrating the graphite furnace after optimization 65
2.5.7 Determination of cadmium using the newly developed furnace program 65
2.6 The VGA-77 with AA module used for this research 66
2.6.1 The operating conditions and specifications of VGA-77 with AA module for mercury determination 67
2.6.2 Calibrating the VGA-77 with mercury standard solutions in 2% v/v HNO3 68

2.6.3 Calibrating the VGA-77 with standard solutions matrix matched to the samples 69
2.6.4 Spiking the samples for method validation of the VGA-77 AA system 70
2.6.5 VGA-77 ICP-OES system: An alternative to the VGA-77 AA system 70
2.7 The VGA 77 - ICP system used for the current research 71
2.7.1 The operating conditions and specifications of the VGA-77 with ICP-OES module 72
2.7.2 Calibrating the VGA-77 ICP-OES system mercury standard solutions 73
2.7.3 Spiking the samples for method validation of the VGA-77 ICP-OES system 73
CHAPTER 3 74
3. Results and Discussion 74
3.1 Determination of Solids content in the sewage sludge 76
3.2 The sources of uncertainty in the acid digestion 78
3.3 Quantifying the uncertainty in the calibration of the ICP-OES 80
3.3.1 The volume of the volumetric flask (V) 80
3.4 ICP-OES reproducibility and contribution to uncertainty budget 83
3.5 Quantifying the uncertainty in the sub-sampling 84
3.5.1 The uncertainty in weighing for the sub-sampling techniques 84
3.6 Quantifying the uncertainty on Sieved & Ground samples 85
3.6.1 Comparing the precision between coned & quartered against random samples 89
3.7 Quantifying the uncertainty in non-sieved & ground samples 90
3.7.1 Comparing the precision of coned & quartered samples against non-sieved & non-ground random samples 92
3.7.2 Comparison of overall precision using the F-test 93
3.8 Calculating the overall uncertainties 94
3.9 Selection of the best method for cadmium analysis 97
3.9.1 The determination of cadmium using the graphite furnace 98
3.9.2 GFAAS reproducibility and contribution to uncertainty budget 98

3.9.3 The F-test to compare the difference between the ICP-OES & GFAAS reproducibility 99
3.9.4 GFAAS determination of the cadmium before optimization 99
3.9.5 Determination of Cd using the optimized furnace program 101
3.9.6 The overall uncertainty in the determination of cadmium was calculated using equation 6 as follows: 102
3.10 Determination of mercury in the VGA-77 AAS system 103
3.10.1 VGA-77 AAS reproducibility and contribution to uncertainty budget 103
3.10.2 Quantifying the uncertainty in the random subsampling for the VGA-77 AAS 104
3.10.3 Comparing the repeatability of the VGA-77 AAS against random subsamples 104
3.10.4 The overall uncertainty of the mercury concentration using the VGA-77 AAS system 105
3.10.5 The effect of nitric acid concentration in the determination of mercury 105
3.10.6 The overall uncertainty of the mercury concentration using the VGA-77 AAS system for matrix matching: 107
3.11 Determination of mercury in the VGA-77 ICP-OES system 108
3.11.1 VGA-77 ICP-OES reproducibility and contribution to uncertainty budget 108
3.11.2 Quantifying the uncertainty in the random subsampling for the VGA-77 ICP-OES 109
3.11.3 Comparing the VGA-77 ICP-OES repeatability precision against random subsamples 110
3.11.4 The overall uncertainty of the mercury concentration using the VGA-77 ICP-OES system was calculated as follows: 110
3.11.5 Mercury recovery on the VGA-77 AAS against the VGA-77 ICPOES system 111
3.12 Overall summary of errors and comparison with literature data 113
3.13 Predicting precision of the detection systems using the Horwitz equation 122
CHAPTER 4 124
4. Conclusion 124
5. References 127
Appendix 134

LIST OF FIGURES

Figure 1: The potential different distributions of the inorganic and organic contaminants (Dean, 2003) .. 6
Figure 2: A schematic diagram for the sampling process (Mitra & Brukh, 2003) 7
Figure 3: The basic sampling methods for environmental analysis (Dean, 2003) 8
Figure 4: The uncertainty estimation process (EURACHEM, 2012) 14
Figure 5: The Fishbone diagram – Structure (Ilie & Ciocoiu, 2010) 15
Figure 6: Relationship between LoB, LoD and LoQ (Armbruster & Pry, 2008) 19
Figure 7: The different systems of atomic spectroscopy (Boss & Fredeen, 1997) 26
Figure 8: The acid digestion of the sewage sludge samples (ISO 11466) 37
Figure 9: The VARIAN 720-ES ICP-OES instrument used for this research 38
Figure 10: The calibration curve for the arsenic 52
Figure 11: The calibration curve for the chromium 52
Figure 12: The calibration curve for the lead 53
Figure 13: The calibration curve for the cadmium 53
Figure 14: The THGA Graphite Furnace instrument used for this current research 55
Figure 15: Calibration of the GFAAS before process optimization 58
Figure 16: The absorption peak for the standard solution at 800/1400 ^{o}C 60
Figure 17: The absorption peak for the standard solution at 900/1400 ^{o}C 60
Figure 18: The absorption peak for the standard solution at 1000/1400 ^{o}C 61
Figure 19: The absorption peak for the standard solution at 1000/1500 ^{o}C 61
Figure 20: The absorption peak for the spiked sample at 800/1400 ^{o}C 62
Figure 21: The absorption peak for the spiked sample at 900/1400 ^{o}C 63
Figure 22: The absorption peak for the spiked sample at 1000/1400 ^{o}C 63
Figure 23: The absorption peak for the spiked sample at 1000/1500 ^{o}C 64
Figure 24: Calibration of the GFAAS after process optimization 65
Figure 25: The VGA-77 for cold vapour generation of mercury with installed AA module 66
Figure 26: The calibration curve for the mercury standard solutions prepared in 2% v/v HNO_3 68
Figure 27: The calibration curve of matrix matched mercury standard solutions 69
Figure 28: The VGA – 77 with installed ICP-OES module 71
Figure 29: The calibration curve for the mercury standard solutions 73

Figure 30: The cause and effect (Ishikawa) diagram for the determination of metal concentrations..... 75
Figure 31: Column chart diagram showing the sampling errors obtained from the techniques 94
Figure 32: The interference observed in the determination of the cadmium..... 97
Figure 33: The cause and effect (Ishikawa) diagram for the determination of metal concentrations..... 124

LIST OF TABLES

Table 1: The different types of acids mostly used for wet decomposition techniques 22
Table 2: The summary of the strengths and limitations of the instruments for chemical analysis 28
Table 3: The different wavelengths of the arsenic and their respective intensities 41
Table 4: The different wavelengths of the cadmium species and their respective intensities 42
Table 5: The intensity of the wavelength line chosen for Chromium 42
Table 6: The wavelengths and intensities of the potential interferences of the lead 43
Table 7: The wavelengths to be used for each of the metal pollutants in the ICP-OES 43
Table 8: The concentrations of the analytes in the standards used for calibrating the ICP-OES 44
Table 9: The current furnace program used before optimization 57
Table 10: Standard solution absorbance readings during the optimization of the furnace program 59
Table 11: Spiked sample absorbance readings obtained during the optimization of the furnace program 62
Table 12: The masses of the digested sewage sludge samples 65
Table 13: Solids content of the sewage sludge from oven drying at 100 ^{o}C 76
Table 14: Estimating the uncertainty in the weighing for solids content determination 78
Table 15: The impurities in the acids used for aqua regia digestion (Merck Millipore, 2016) 79
Table 16: The relative standard uncertainty in the volumetric flask 83
Table 17: The values and uncertainties in the Instrument's reproducibility 83
Table 18: Estimating the uncertainty in the weighing of random sampling 84
Table 19: The concentrations of the analytes obtained from coned & quartered subsamples 85
Table 20: Comparing precision of the ICP-OES reproducibility against coning & quartering using F-test 86
Table 21: The concentrations of the analytes obtained from the random sampling 87
Table 22: Comparing relative precision of the ICP-OES reproducibility against random sampling using F-test 88
Table 23: Comparing relative precision of the coning & quartering against random sampling using F-test 89
Table 24: Analyte concentrations from the random sampling of non-sieved & non-ground samples 90
Table 25: Comparing the relative precision of the ICP-OES reproducibility against non-sieved & non-ground sub-sampling using F-test 91
Table 26: Comparing precision of the sieved & ground against non-sieved & non-ground samples 92
Table 27: Comparing precision of the sieved & ground against non-sieved & non-ground samples 93

Table 28: Listing all the sub-sampling errors for the analytes 94
Table 29: Reporting the expanded uncertainties for sieved and ground, cone and quartered samples ... 95
Table 30: Reporting the expanded uncertainties for sieved and ground random samples 96
Table 31: Reporting the expanded uncertainties for sieved and ground coned and quartered samples .. 96
Table 32: The reproducibility experiment of the cadmium using a 10 µg. L^{-1} standard 98
Table 33: The F-test to compare the precisions of the ICP-OES & GFAAS for determining cadmium..... 99
Table 34: Cadmium concentrations of the random samples before optimization..... 100
Table 35: Cadmium concentrations of the random samples after a new furnace program 101
Table 36: The repeatability experiment to determine the standard uncertainty of the mercury 103
Table 37: Mercury concentrations obtained from the random sampling for the VGA-77 AAS..... 104
Table 38: The F-test to compare the precisions of the VGA-77 AAS against random subsamples 104
Table 39: Mercury concentration in the random subsamples for matrix matched calibration of the VGA 77 – AAS 106
Table 40: The F-test to compare the precisions when the effect of nitric acid is assessed 107
Table 41: The repeatability experiment to determine the standard uncertainty of the mercury 108
Table 42: The F-test to compare the precision of the VGA-77 AAS against VGA-77 ICP-OES 109
Table 43: Mercury concentrations obtained from the random sampling for the VGA-77 ICP-OES 109
Table 44: The F-test to compare the precisions of the VGA-77 ICP-OES against random subsamples 110
Table 45: The recovery experiment for the mercury on the VGA-77 AAS system 111
Table 46: The recovery experiment for the mercury on the VGA-77 ICP – OES system 112
Table 47: Errors associated with each part of the procedure for the determination of arsenic..... 113
Table 48: The South African wastewater sludge classification system (Snyman & Herselman, 2006) 114
Table 49: Metal limits for South African Wastewater sludges (Snyman & Herselman, 2006) 115
Table 50: Results of arsenic analysis in sewage sludge found in literature 116
Table 51: Errors associated with each part of the procedure in the determination of the cadmium 116
Table 52: Result of cadmium analysis in sewage sludge samples found in literature 117
Table 53: Errors associated with each “cause” for the determination of chromium in the ICP-OES 118
Table 54: Result of chromium analysis in sewage sludge samples found in literature 119
Table 55: Errors associated with each “cause” for the determination of lead in the ICP-OES..... 119
Table 56: Result of lead analysis in sewage sludge samples found in literature 120
Table 57: Errors associated with each “cause” for the determination of Mercury 121

Table 58: Comparing the precision of the concentrations from our research with the Horwitz prediction 122

LIST OF EQUATIONS

Equation 1: The model for uncertainty combination involving only a sum of quantities……77
Equation 2: The estimation of uncertainty using a triangular distribution……81
Equation 3 : The estimation of volume variation (Eurachem, 2012)……81
Equation 4: The estimation of uncertainty using a rectangular distribution……82
Equation 5: The estimation of the uncertainty of the volume of the flask ……82
Equation 6: The calculation for overall combined uncertainty ……95
Equation 7: The Horrat equation for calculating the Horrat value…… ….122

CHAPTER 1

1. Introduction

1.1 Sewage sludge

Sludge is the residual, solid material removed or left behind during the treatment of wastewaters and sewage treatment processes. There are three different kinds of sludges, the first one is Sewage sludge from municipal treatments works (e.g. Umgeni Darvill Waste Water Works), Septage which is pumped from septic tanks and lastly, Industrial sludges (Hope, 1986). Since the main focus of this work will be on sewage sludge from municipal treatments, it is appropriate to briefly discuss its generation as well as the various forms it can be obtained in.

1.1.1 Generation of sewage sludge and its various forms

Water is used on the daily basis worldwide and some of it ends up in the sewage system and from there it is channeled to sewage treatment plants (Wiechmann *et al.,* 2011). At these plants, the sewage passes through screens and sieves and undergoes mechanical and biological purification. The goal of such treatments and purification is to remove impurities from the sewage and to then channel the resulting purified water into water bodies. The residue of this entire process is known as 'sewage sludge' which can occur in anhydrous, dried or other processed forms (Wiechmann *et al.,* 2011). There are various forms at which sewage sludge can be obtained in and these are discussed briefly below.

As it has been mentioned above that sewage sludge is generated by both municipal and industrial sewage treatment plants, there is also raw sludge which is sewage sludge removed from sewage treatment plants without being treated. Raw sewage sludge comprises of primary, second and tertiary sludge in any given mixture that occurs at a sewage treatment plant and it is untreated prior to stabilization (Wiechmann *et al.,* 2011). Primary sludge occurs in the mechanical preliminary treatment phase and therefore results from the physical process used to filter particulate substances out of wastewater. Primary sludge is often greyish black to yellow in color and contains debris such as toilet paper (Wiechmann *et al.,* 2011).

Secondary sludge (also referred to as surplus activated sludge) occurs during biological treatment and it is generated by microbial growth. It has a brown colour and more homogenous than primary sludge. When the secondary sludge is removed from the treatment system, it is digested more rapidly than is the case with primary sludge (Wiechmann *et al*., 2011).

The sludge that occurs in municipal sewage treatment plants resulting phosphate precipitation (i.e. removing phosphorous from a solution using iron salt or lime) is known as tertiary sludge. Tertiary sludge often occurs mixed in with primary or secondary sludge and it is normally stable and does not emit an unpleasant smell (Wiechmann *et al*., 2011).

1.1.2 Disposal of sewage sludge

The Environmental Protection Agency in the United States of America lists the following ways to dispose of sewage sludge:

- Application of sludges to agricultural and non-agricultural lands
- Distribution and marketing of sludges which is basically the sale or give-away of sludge for use in home gardens
- Disposal in municipal (sludge only) landfills and surface disposal sites
- Incineration of sludge (Environmental Protection Agency, 1993)

1.1.3 Benefits of sewage sludge

Previously, sewage sludge used to be dumped on land without understanding the extent of the impact it imposed in the environment until regulations were set in many countries to forbid this action. In the 1980s and 1990s a large worldwide research campaign to investigate the potential benefits of using sludges as forest fertilizers was initiated (Moffat, 2006). The main outcome of this research was the manual titled "*A manual of good practice for the use of sewage sludge in forestry*" by (Wolstenholme *et al*., 1992) which discusses in-depth when and how liquid and cake sludges might be used to increase forest productivity on infertile sites (Moffat, 2006).

Recent studies have shown that the application of sewage sludge to agricultural lands provides multiple agronomic benefits. Some of these benefits include the recycling of plant nutrients such as nitrogen and phosphorus thus making sludge an effective replacement for chemical fertilizers. The most recognized environmental benefit is the recycling of phosphorus (P) in the food chain which subsidizes the conservation of mineral phosphorus reserves and also reduces external inputs of cadmium (Cd) present in phosphate rocks. Moreover, sludge also offers other macronutrients such potassium (K) and sulfur (S) and micronutrients such as copper (Cu) and zinc (Zn).

Sewage sludge has not only been used extensively on agricultural land but it has also been successfully utilized in the fields of forestry and in land reclamation operations like closed landfills (Milieu *et al.,* 2008). Lastly on the benefits of sewage sludge; the application of sewage sludge in agriculture replaces the use of chemical fertilizer and the nitrous oxide (N_2O) emissions associated with that fertilizer are considered to be a negative emission (Milieu *et al.,* 2008). "If all of the nitrogen in the sludge were available to plants the N_2O emissions from the soil after application would be balanced by the reduced N_2O emissions from the chemical fertilizer. In fact as little as 20% of the nitrogen in digested sludge cake is considered to be readily available to plants so the emissions of N_2O from its spreading are greater than the reduction in N_2O from the displaced fertilizer" (Milieu *et al.,* 2008).

1.1.4 Concerns of sewage sludge

Although sewage sludge contains several nutrients as well as organic matter that are beneficial for the soil, it also contains some contaminants such as heavy metals, pathogenic bacteria, viruses, protozoa, parasites, worms and organic compounds. The presence of nitrogen in the sewage sludge can be problematic if there is no proper management and if it is in excess it can contaminate groundwater or surface waters (Hope, 1986). The presence of heavy metals and in particular, cadmium, is a serious problem in sewage sludges globally and so these contaminants must be carefully managed for the safety of the public and integrity of the environment (Milieu *et al.,* 2008).

1.1.4.1 Heavy metals

There is no definitive meaning for the term heavy metals but nevertheless these metals are described as members of a subset of elements that show metallic properties and include mostly transition metals, a few metalloids, lanthanides and actinides. There are various other definitions of the term 'heavy metal' based on density, chemical properties and toxicity (Duffus, 2002). Lide defined heavy metals as "Chemicals with a specific gravity that is at least five times the specific gravity of water" (Lide, 1992). This definition enables arsenic (metalloid) as well as lead, mercury, cadmium & chromium to be classified as heavy metals since their specific gravities are in line with the definition. The presence of heavy metals in sewage sludge is the most problematic when land application is to be implemented. Recent research has been dedicated and focused on finding ways to manage the presence of heavy metals in sewage sludge.

In a study conducted by Hope, 1986 it was shown that the metals which are of high concern in soils augmented with sewage sludge are zinc (Zn), copper (Cu), nickel (Ni), mercury (Hg), arsenic (As) and most especially cadmium (Cd) (Hope, 1986).

Cadmium, lead, mercury and arsenic have been shown to be toxic to humans while the toxicity of zinc, copper and nickel has been reported to plants. A prolonged exposure to lead, mercury and arsenic may cause chronic poisoning of animals and human beings including disabilities and even death (Hope, 1986). Recent research conducted at the University of Aberdeen by Feldmann *et al.,* 2015 for the detection of inorganic arsenic in rice has shown that market bought rice in Aberdeen revealed that levels of inorganic arsenic from rice produced in the USA were much higher than that in rice from India and Bangladesh.

This research from the University of Aberdeen received a lot attention and has become influential in the studies of the levels of arsenic in rice. Much of this attention comes from the fact that old literature (e.g. Hope, 1986) suggested that plants do not take up cadmium, lead, mercury and arsenic in any quantity and that these metals can never be transported to edible parts of the plants. All of these claims from old literature have been shown not to be accurate anymore thereby suggesting that the consumption of plant matter grown from sludge amended soils might impose harm to the people or animals.

The effects of chronic poisoning by lead, mercury and arsenic have been well studied and characterized and they include behavioral problems, damage to the brain, poor learning and depression (Hope, 1986). Contamination and dirt (i.e. pollution) are the most important sources of danger for human health that may directly enter the food chain rather than through plant uptake. This may occur as a result of spraying sludge in liquid form on the growing crops as it may become dry and strongly adhere to the plants and become difficult to wash off even by heavy rainfall (Hope, 1986).

Metals from the soil may also be absorbed onto plant roots including edible roots such as carrots and radishes. Grazing animals that ingest up to as much as 30 % of the soil as part of their diet also provide another pathway for entry of the heavy metals into the food chain. This means that heavy metals such as zinc, copper and nickel have a possible chance of entering the human pathway. These metals mount up on the liver, kidney and the bones of the animals that ingest the contaminated plants (Hope, 1986).

Cadmium is discussed separately because this metal shows both a chronic and an acute poison. The accumulation of cadmium in the kidneys results in irreversible kidney damage, urinal system problems and many more diseases (Hope, 1986). Cadmium has been shown to be taken up by plants from the roots up to their leaves (e.g. spinach and carrots) and this occurs mostly at an acidic pH level of 6.5. Reports

from the 'World Health Organization' have shown that there has been a dramatic increase of the cadmium concentrations in soils and human diets for many years (Hope, 1986).

1.1.4.2 Pathogenic organisms

It has been mentioned previously that sewage sludge also contains infectious pathogenic organisms like bacteria, viruses, protozoa and parasites and some of these pathogens can be killed by treatment processes like heating sludge and composting especially in sludge that is to be sold to consumers. Since the treatment processes kill only up to 99 % of the harmful bacteria in the sewage sludge, this means that the remaining 1% still has the potential of causing diseases. Viruses (e.g. hepatitis A) are also found in sludge and due to their smaller sizes their occurrence is greater than that of bacteria. The small sizes of the viruses make them difficult to monitor in the environment and also difficult to culture at the laboratories for research studies (Hope, 1986).

1.1.4.3 Toxic organic substances

This is a group of organic substances that have adverse effects on the environment as well as public health e.g. persistent pesticides. The mode of transportation of these substances through the environment together with the monitoring techniques is very little known. However, upon the treatment of sewage sludge most of these substances are inactivated (i.e. they are volatilized into air). Nevertheless certain toxic organics like Polychlorinated biphenyls (PCB's) are highly persistent (Hope, 1986).

1.1.5 Composition of sewage sludge

Sewage sludge is regarded as a multi-substance mixture and because of the inhomogeneity and remarkable differences in the concentrations of the various components, it is difficult to determine or define a standard composition for sewage sludge (Wiechmann *et al.,* 2011). Sewage sludge contains various components such as heavy metals, therefore it is very important to monitor levels of these heavy metals and an important part of this analysis is appropriate sampling and choice of analytical methodology.

1.2 Sampling

1.2.1 Description of sampling and its importance

Sampling is defined as the process that allows one to obtain a representative picture about the population without studying it entirely (Molenberghs, 2004). If it were feasible, all sample measurements would be done and analysed directly in the field without the need of shipping the samples to the laboratory. However that would be an impossible task to do and so in order to cut down the analysis time, cost and the efforts, the areas to be investigated must be sampled (Dean, 2003).

There are two main types of sampling for environmental samples; random sampling and purposeful sampling, the former being the most important because it infers no selectiveness to the sampling process. By inferring no selectiveness this means that the process is completely randomized and as a result bias is eliminated (Dean, 2003).

All environmental sampling procedures involve the careful selection of the sample points, the size and shape of the sample area as well as the sum of the sampling components in each of the samples. The information about the distribution of the contaminants (e.g. inorganic, organic and pathogenic) must be known and these contaminants may be distributed in any of the ways shown in figure 1 below (Dean, 2003).

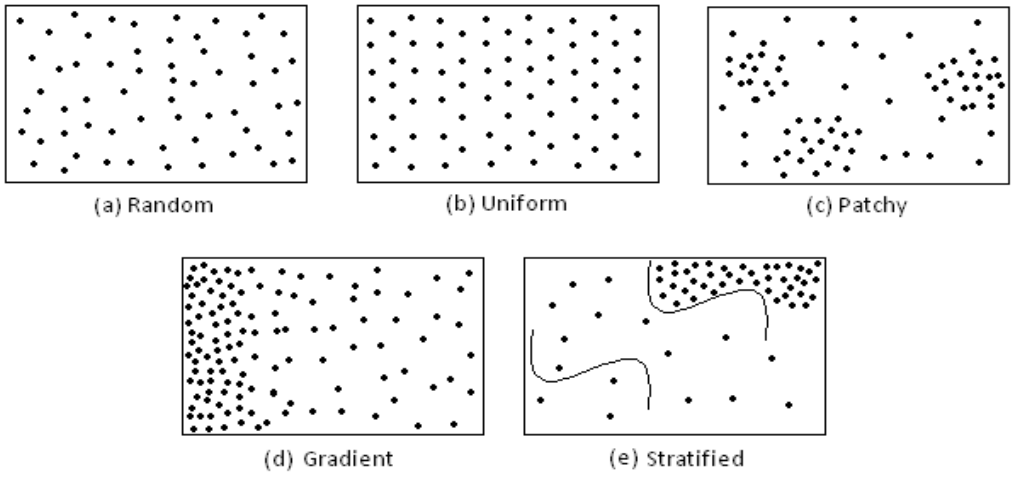


Figure 1: The potential different distributions of the inorganic and organic contaminants (Dean, 2003)

As well as spatial variability you may also have temporal variability in the sample. For example when collecting samples for the determination of Ca^{2+} in a lake, it should always be kept in mind that the concentrations may be different depending on various factors like location, depth and season of the year (Mitra & Brukh, 2003). There is no objective way of determining how far away from or close to the population parameter the estimation made may be. For example, finding Ca^{2+} concentration on the lake samples only gives you an estimate and does not account for the entire lake (i.e. the population). This demonstrates that even in bodies of water which have a decent mixing, one can still get variation and this is expected to be worse in solids (Mitra & Brukh, 2003).

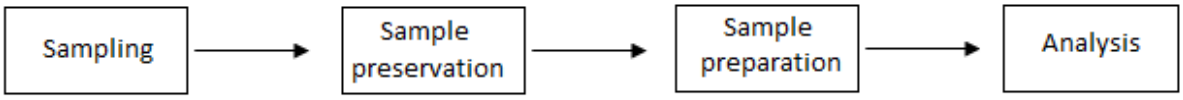


Figure 2: A schematic diagram for the sampling process (Mitra & Brukh, 2003)

1.2.2 Sampling Techniques

There are various types of sampling techniques each having their own unique application (refer to figure 3 for examples). Ideally, simple random sampling is often the most preferred technique by researchers but due to time constraints or the lack of resources to access the whole population, alternatives to random sampling have to be considered.

1.2.2.1 Non-probability random sampling

This is a sampling technique which doesn't make use of samples with known probabilities of selection, hence random. Random sampling is used when access to the entire population is possible. Whenever the process of sample selection is not performed randomly, the sampling results may be biased. When the results are biased the average value of the estimate in the repeated sampling is not equal to the parameter that is being investigated. In simple terms, this means that the sample may not be a true representative of the population of interest (Aczel & Sounderpandian, 2009).

1.2.2.2 Stratified random sampling

There are cases where the population may be viewed as comprising of different groups where the components of each group are related to one another in some way. In such cases, it is possible to attain the precision of the sampling as well as reduce the costs of the survey by treating the different groups separately. Such groups are referred to as *strata* and if they are dealt with as separate subpopulations by drawing separate random samples from each stratum, that sampling method is called 'stratified random sampling' (Aczel &Sounderpandian, 2009).

1.2.2.3 Cluster sampling

There may be cases where there is no clear description of all the components which may possibly be present in the population due to them 'clustered' in larger units (refer to the "Patchy" diagram in the figure 1 above). Within these clusters or units there are several components of the population and therefore using the method of 'cluster sampling' will be fitting (Aczel &Sounderpandian, 2009).

1.2.2.4 Systematic sampling

In this type of sampling the position of the first sample is taken at random and then taking more samples at fixed directions from this. This type of sampling technique provides results which are more accurate than a simple random sampling. However, biased samples may result if the soil comprises of a systematic variation that is coincidental with this type of sampling (Dean, 2003).

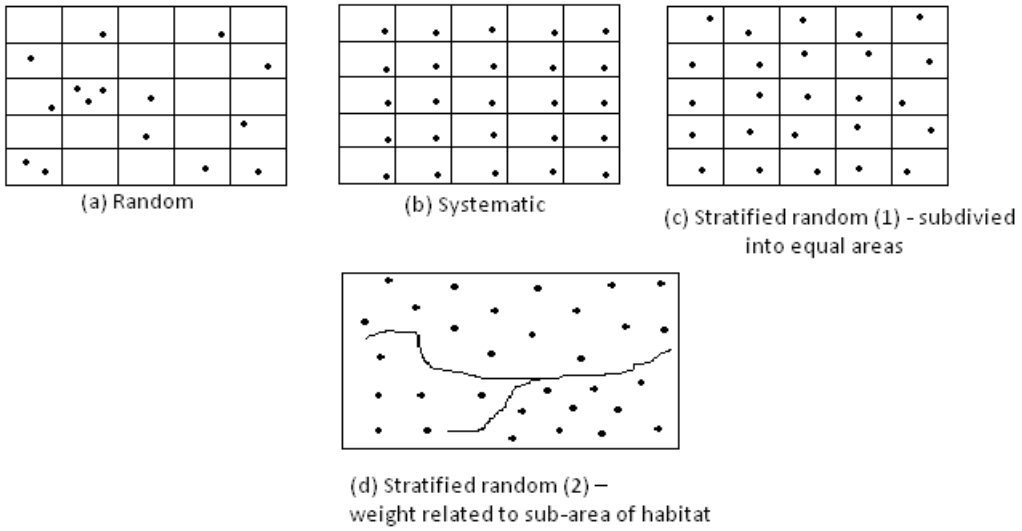


Figure 3: The basic sampling methods for environmental analysis (Dean, 2003)

1.2.3 The preservation of samples

Once the sampling is done the next step is to store and preserve these samples before transporting them (in the same state) to the laboratory for analysis. The sample storage and preservation methods utilized should be able to inhibit any chemical, physical or biological changes that may alter the integrity of the sample. “Therefore the ultimate goal that one must strive for is to keep the shortest time interval between the sampling and the analysis” (Dean, 2003).

1.3 Estimation of sampling errors and the effect of particle size

1.3.1 Errors in analysis

All measured physical quantities are susceptible to a measurement error i.e. no physical quantity can be measured with perfect certainty. In most cases error is frequently confused with uncertainty when in actual fact the two are defined very differently from one another. Error is defined as the difference measured between an individual result and the true value (EURACHEM, 2012). Therefore through knowing the true value, one can calculate an error which will be a single value and this value can also be used to judge the accuracy of the measurement. It also worth mentioning here than an error is just an idealized concept since it can never be known exactly (EURACHEM, 2012).

Therefore, whenever a measurement of a quantity is made and then repeated, one will almost certainly measure a different value the second time therefore suggesting that errors are always inevitable. However, as greater care is taken in the measurements and more redefined experimental methods are applied, errors can then be reduced. The greater number of repetitions increases reliability and precision and thereby help us attain the confidence that our measurements are closer to the true value (EURACHEM, 2012).

Errors are divided into several types including experimental errors, systematic errors, random errors & calculating experimental errors and these are explained below in simplistic terminology:

- Experimental error: “Error is defined as the difference between an individual result and the true value of the measurand. In practice, an observed measurement error is the difference between the observed value and a reference value. As such, error – whether theoretical or observed – is a single value. In principle, the value of a known error can be applied as a correction to the result. NOTE: Error is an idealized concept and errors cannot be known exactly” (EURACHEM, 2012).
- Systematic error: These affect the accuracy of the measurement and are one sided and persistent regardless of how many times a measurement is being repeated. Common sources of systematic errors are faulty calibration of measuring instruments, poorly maintained instruments, or faulty reading of instruments by the user.
- Random errors: These affect the precision of a measurement and are two sided because repeat measurements fluctuate above and below the true value. To reduce them, several repeat measurements must be made or by refining the measurement method or technique.

1.3.2 Particle size analysis

The distribution of particle size is often used in the estimation of soil moisture characteristics or hydraulic conductivity, unsaturated conductivity and various other purposes e.g. nutrient acquisition (Anderson *et al.,* 2006). Determination of particle size distribution by sieving, hydrometer and pipette methods or by laser diffraction suffers from many short comings due to the difficulty of the size of irregularly shaped particles (Eshel *et al.,* 2004).

Jakab *et al.,* 2010 conducted a particle size distribution study for the analysis of different samples using different methods. The aim of their study was to prove if there was or wasn't a difference in the particle size determined using different samples and different methods. The methods they used are shown below.

- "Aerometer method: the method is based on measuring the density of soil suspension at different times during Sedimentation. The aerometer method was used by the University of Szeged" (Jakab *et al.,* 2010).
- Pipette method: samples are taken from the settling soil suspension at 5 different times with a pipette. Samples are dried and weighed then the different fractions are calculated based on Stokes' law. The pipette method was used by the University of Szeged, Debrecen and West Hungary (Jakab *et al.,* 2010).
- "Laser method: the Geographical Research Institute of the Hungarian Academy of Sciences used the laser method to measure particle sizes with Laser Particle Sizer Analysette 22 MicroTec" (Jakab *et al.,* 2010).

The main finding of this study was that different analytical methods produced different results for particle size distribution. Moreover, the analysis of variance revealed significant different probability values and such results can be helpful in seeking sources of errors. There was also an in discrepancy observed in that the analyses repeated with one method had less than 5% error while another had more than 20%. From there it was concluded that not only do the well-known differences between methods, sample preparation and physical background matter but there are other factors (e.g. routine vs closely checked analyses) which may influence the results (Jakab *et al.,* 2010).

1.3.2.1 Sieve analysis

Sieve analysis is the oldest, best known and widely used method for particle size determination. The distribution of the particle size on this method is defined via the mass or volume. Sieve analysis is used to section the granular material into different fractions and then to determine the weight of these fractions. In this manner, a broad spectrum of the particle size can be analyzed. During sieve analysis the sample is placed on the sieve and then subjected to either horizontal or vertical movement which causes the movement between the particles and the sieve (Grzechnik & Pitsch, 2004). The individual particles can either pass through the sieve mesh or be retained on the surface. The ratios of the particle size to the openings of the sieve, the mesh size of the sieve, the quantity of material on the sieve surface as well as the orientation of the particles are some of the factors that determine the possibility of a particle passing through the sieve mesh (Grzechnik & Pitsch, 2004).

1.3.3 Measurement uncertainty & Uncertainty budget

Uncertainty is defined as a parameter (e.g. a standard deviation or any multiple of it) associated with the result of a measurement which characterizes the dispersion of the values that could be attributable to the measurand (a physical quantity, property, or condition which is measured) (EURACHEM, 2012). The uncertainty of measurement comprises of a variety of components which may be evaluated from the statistical distribution of the results of a series of measurements and can be illustrated by standard deviations (EURACHEM, 2012).

There are many possible sources of uncertainty for the results obtained from analytical measurements and some of these include; the employed sampling technique, storage conditions, instrument conditions, reagent purity, assumed stoichiometry, matrix effects and interferences, environmental conditions and random variation (EURACHEM, 2012). In the estimation of the overall uncertainty, each source of uncertainty must be taken and treated separately in order to obtain the contribution from that source. These separate contributions to uncertainty are each referred to as an ‘uncertainty component’ and when expressed as standard deviations, each is known as a ‘standard uncertainty’. If however there is a correlation between any of the components then this has to be taken into account by determining the covariance (EURACHEM, 2012).

The following paragraphs summaries the tasks that need to be performed in order to obtain an estimate of the uncertainty associated with a measurement result.

1.3.3.1 The process of measurement uncertainty estimation

There are four main steps involved in the process of measurement uncertainty estimation and these are:

a) Specifying the measurand
b) Identifying uncertainty sources
c) Quantifying uncertainty components
d) Calculating combined uncertainty

Specifying the measurand means writing down what is being measured as well as the relationship between the measurand and the input quantities (e.g. measured quantities, calibration standard values) with which it depends upon. If systematic effects are known, these must be corrected if possible and the specification information should be given in a Standard Operating Procedure (EURACHEM, 2012). The next step is identifying uncertainty sources and this includes listing all possible sources of uncertainty which could be coming from the parameters in the relationship between the measurand and input quantities. The general representation of the uncertainty sources and their contributions is through the use of cause and effect (Fish bone/Ishikhawa) diagrams (EURACHEM, 2012), which will be discussed in the section below.

The third step is to quantify the size of the uncertainty component associated with each potential source of uncertainty identified. Determining a single contribution to uncertainty associated with other separate sources is possible when data from validation studies or quality control is used. Using this data significantly reduces the effort needed to evaluate the uncertainty and since actual experimental data is used, this can lead to reliable estimates of the uncertainty (EURACHEM, 2012).

The last step is calculating the combined uncertainty by using all the quantified contributions to overall uncertainty obtained in the third step. These contributions are expressed as standard deviations and combined according to the appropriate rules to give a combined standard uncertainty and the appropriate coverage factor is applied to give an expanded uncertainty. Figure 4 shows schematically the whole process of measurement uncertainty estimation (EURACHEM, 2012).

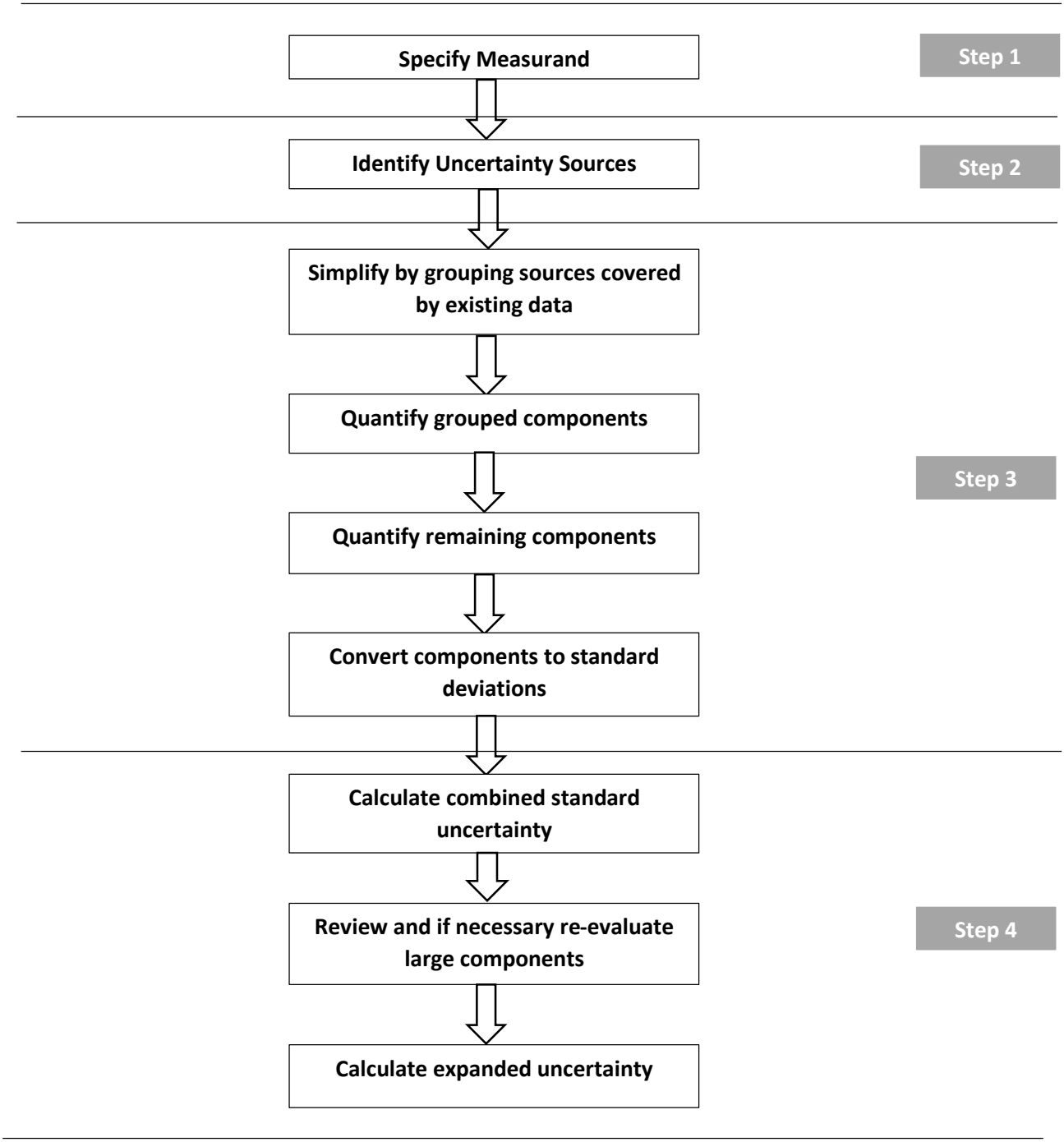


Figure 4: The uncertainty estimation process (EURACHEM, 2012)

1.3.3.2 The importance & use of Fishbone diagrams

The Fishbone diagram (also known as the cause & effect or Ishikawa diagram) is a tool used for analyzing process dispersion and/or identifying the root causes of quality problems. It was named after Kaoru Ishikawa, a Japanese quality control statistician, the man who pioneered the use of this chart in the 1960's (Ilie & Ciocoiu, 2010). The Ishikawa diagram is referred to as a Fishbone due to its resemblance of a fish skeleton. In a Fishbone diagram, the effect is often the problem that needs to be resolved and it is placed at the "fish head" and the causes are laid out along the "bones" and classified into different types along the branches. The rest of the causes can be laid out alongside further side branches to give a general structure as depicted in figure 5 (Ilie & Ciocoiu, 2010).

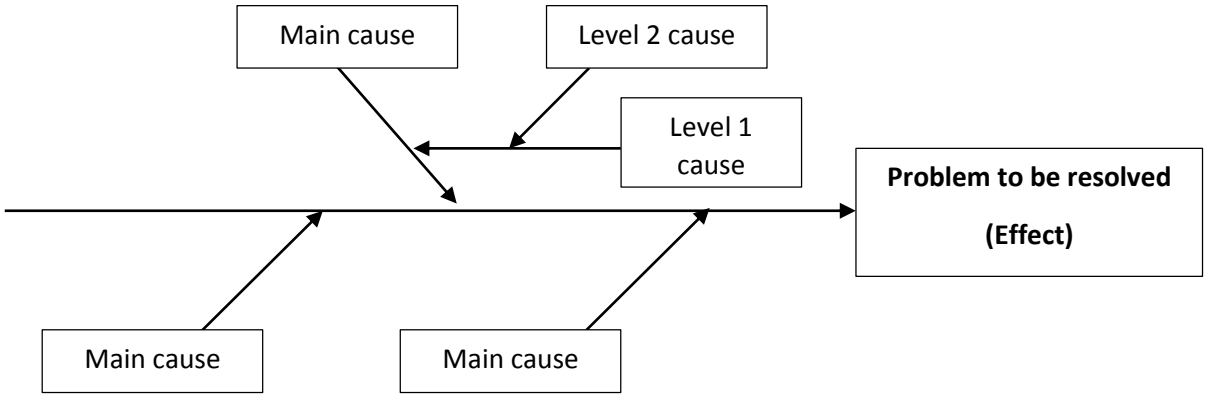


Figure 5: The Fishbone diagram – Structure (Ilie & Ciocoiu, 2010)

There are several benefits of using the Fishbone diagram and these include getting an aid in the determination of root causes. Moreover, the orderly, easy-to-read format is used to diagram cause and effect relationships and the possible cause of variation is indicated. Lastly, areas for collecting data are indicated. For example, if "calibration" has been listed as a "Main cause" then a starting point for data collection would be at "Level 1" and subsequently "Level 2" (Ilie & Ciocoiu, 2010).

1.4 Parameters and tests for method validation

"In practice, the fitness for purpose of analytical methods applied for routine testing is most commonly assessed through method validation studies. Such studies produce data on overall performance and on individual influence factors which can be applied to the estimation of uncertainty associated with the results of the method in normal use" (EURACHEM, 2012). The studies of method validation depend on the determination of the overall method performance parameters obtained during the development of the method and interlaboratory validation protocols (EURACHEM, 2012).

The individual sources of uncertainty are investigated only when significant compared to the overall precision measures in use. "The emphasis is primarily on identifying and removing (rather than correcting for) significant effects. This leads to a situation in which the majority of potentially significant influence factors have been identified, checked for significance compared to overall precision, and shown to be negligible" (EURACHEM, 2012). Under these conditions, the data available to analysts is primarily of overall performance figures, the evidence of insignificance of most effects and some measurements of any remaining significant effects (EURACHEM, 2012).

Typical validation studies for quantitative analytical methods focuses and determines some or all of the following parameters:

1.4.1 Precision, Accuracy, Bias and Recovery

The accuracy of an analytical procedure is defined as the closeness of agreement between the true value or an accepted reference value and the discovered value. Other definitions state accuracy as the extent of agreement between the test results generated by the analytical method and the true value (Huber, 2010). This true value used for the assessment of accuracy can be obtained in several ways and one of these ways is to assess the accuracy by analyzing a sample with known concentrations and comparing the measured value with the true value as supplied with the material (Huber, 2010). The second way is to compare the results of the method with those from an established method. With this approach there is an assumption that the uncertainty of the reference method is known (Huber, 2010).

The lack of accuracy results into biased measurements and this bias is defined as the total systematic error as contrasted to random error. There may be more than one systematic error components contributing to the bias and a larger systematic difference from the accepted reference value is shown by a larger bias value (Hibbert, 2007). The bias of an analytical method is usually determined by studying the relevant reference materials or by spiking studies and it may be expressed as an analytical recovery (i.e. a value observed divided by a value expected) (EURACHEM, 2012).

If certified reference materials are not available, then a blank sample matrix of interest can be spiked with a known concentration amount. Upon successful extraction of the analyte from the matrix and injection into the instrument for analysis, its recovery can be determined through the comparison of the response of the extract with the response of the reference material dissolved in a pure solvent (Huber, 2010). This recovery will be the proportion or ratio of the concentration of the analyte found to that stated to be present. In general, the results obtained on test materials of the same matrix could, in principle, be corrected for recovery found for the reference material (Thompson *et al.,* 1999). The last remark to make about the assessment of accuracy is that since it measures the effectiveness of sample preparation, extreme care needs to be taken to mimic the actual sample preparation as closely as possible (Huber, 2010).

1.4.2 The working range & linearity

The range of an analytical procedure is defined as the interval from the upper to the lower concentration of analyte in the sample for which it has been shown that the procedure has a suitable level of precision, accuracy and linearity (Huber, 2010). The range is usually expressed in the same units as the test results obtained by an analytical method and these units can, for example be a percentage e.g. parts per million (Huber, 2010). Traditionally, the method is said to be linear when there is a directly proportional relationship between the response of the method and the amount of the analyte in the matrix over the range of analyte concentrations of interest (working range) (UNODC, 2009). Since the working range reflects only a part of the full linear range, a Goodness of Fit test (i.e. a high correlation coefficient (r) of 0.99) is used as an acceptance criterion of linearity. However, this test is not sufficient to prove the existence of a linear relationship and a method with a coefficient of determination of less than 0.99 may still be fit for purpose (UNODC, 2009).

1.4.3 Robustness & Selectivity

The robustness of an analytical technique is defined as a measure of its ability to remain unaffected by small changes in method parameters (Huber, 2010). The robustness also indicates the analytical procedure's reliability during normal usage and also examines the effect that operational parameters have on the analysis results. To determine how robust the method is, a number of parameters such as flow rate, pH, column temperature, injection volume are varied within a realistic range and then the quantitative influence of the variables is determined. If the influence of any individual parameter is said within a previously specified tolerance, then that parameter is said to be within the method's range of robustness (Huber, 2010).

In analytical chemistry the names selectivity and specificity are often used interchangeably and they both relate to the degree to which a method responds uniquely to the required analyte (EURACHEM, 2012). The studies of selectivity investigate the effects of potential interferents by spiking both the blank and fortified samples with this interferent and monitoring the response. These studies measure the changes in response directly, thus making it feasible to use the data to estimate the uncertainty associated with potential interferences (EURACHEM, 2012).

1.4.4 Limit of Blank, Limit of Detection & Limit of Quantitation

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) are terms used to describe the smallest concentration of a measurand that can be reliably measured by an analytical procedure (Armbruster & Pry, 2008).

- Limit of Blank (LoB): This refers to the highest apparent analyte concentration expected to be found when tests are performed on replicates of a blank sample containing no analyte. The calculation for the LoB is, $LoB = Mean_{blank} + 1.645\ (SD_{blank})$ where SD is the standard deviation.
- Limit of Detection (LoD): This is the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible. The limit of detection is measured by using the measured LoB and test replicates of a sample which is known to contain a low concentration of analyte. The calculation of the LoD is, $LoD = LoB + 1.645\ (SD_{\text{low concentration sample}})$.
- Limit of Quantification: This is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met. In some instances the limit of quantitation may be equivalent to the limit of detection or it may even be a much higher concentration (Armbruster & Pry, 2008).

“Typically, the limit of quantitation will be found at a higher concentration than limit of detection but how much higher depends on the specifications for bias and imprecision used to define it” (Armbruster & Pry, 2008). “The slope of the calibration curve is sometimes used as a synonym for LoD. However, because the LoD may be found at some concentration below the linear range of an assay, where the calibration curve is no valid, this usage should be avoided” (Armbruster & Pry, 2008).

The Clinical and Laboratory Standards Institute (CLSI) has published a standard method and guideline EP17 for the determination of Limits of Detection and Limits of Quantitation. The figure 6 shown below is taken from their document and it illustrates the distinction of LoB, LoD and LoQ values. The solid line defines the Limit of Blank (LoB) and represents the distribution of results for a blank specimen (Armbruster & Pry, 2008).

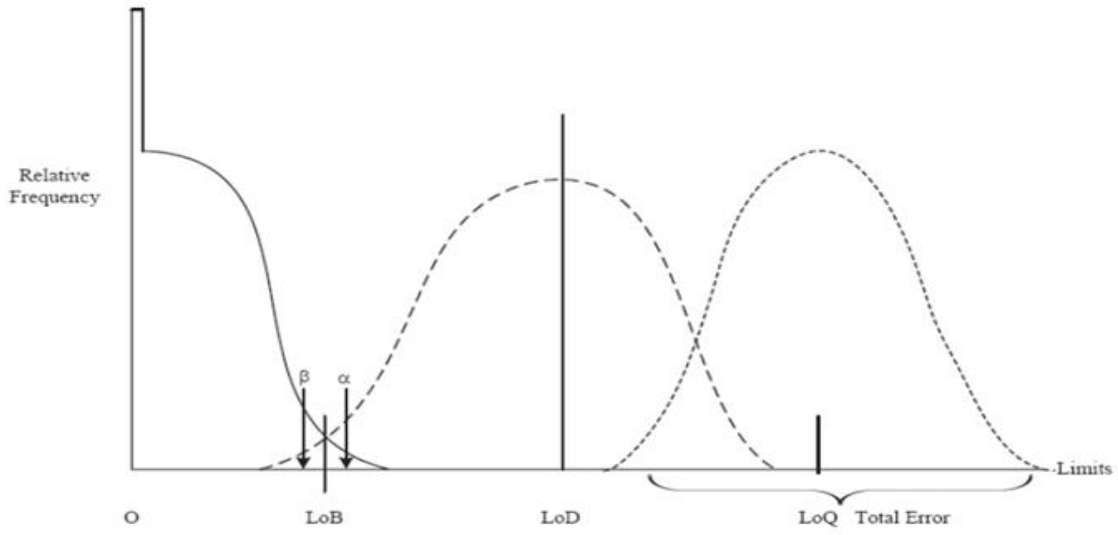


Figure 6: Relationship between LoB, LoD and LoQ (Armbruster & Pry, 2008)

1.4.5 Reference Materials

Reference Materials (RMs) are substances with property values which are sufficiently homogenous and appropriate to be used for the calibration of instruments and/or apparatuses, assessment of an analytical method or assigning values to materials (IAEA, 2003). There are also certified reference materials (CRMs) which only differ from the reference materials in that the former is accompanied by a certificate. The property values of a CRM are certified by a procedure which establishes traceability to an accurate realization to the unit in which the property values are expressed. Each certified value is accompanied by an uncertainty at a stated level of confidence (IAEA, 2003). Reference materials and certified reference materials are used wisely in analytical chemistry and their proper application provides the best information on the quality of the measurement results. These materials have become very important in recent years and absolutely necessary if accuracy of the measurements is to be assessed. Reference materials and certified reference materials are generally prepared in batches either in the form of a pure or mixed gas, liquid or solid and some typical examples include water for the calibration of viscometers and standard calibration solutions in chemical analysis (IAEA, 2003). The composition of these materials should be very close to that of the samples undergoing analysis and in cases of matrix interferences, a matched matrix reference material certified in a reliable manner must be used and if such a material is not available then a sample spiked with a reference material must be used (CITAC, 2002).

Matrix reference materials are another extension of reference materials and they are special because they are natural substances more representative of laboratory samples that have been chemically characterized for one or more elements or constituents with a known certainty (IAEA, 2003). Within reference materials there are different sub-groups, for example gaseous reference materials, environmental and biological matric reference materials, alloys. A large majority of these matrix reference materials is characterized through interlaboratory comparisons. The traceability of these values can be claimed only to the respective laboratory inter comparison and not to any other point of reference (IAEA, 2003). Furthermore, the uncertainty of these values is normally expressed by confidence interval and only gives a measure of the scatter between the laboratory means. Lastly, the uncertainties of the results from individual and independent laboratories are often not regarded and as a consequence matrix reference materials do not always fulfil the criteria for the established traceability of the assigned property values (IAEA, 2003).

1.5 Sample preparation

1.5.1 Preparation of solid samples for inorganic & organic analysis

There are two different techniques that can be carried out for the analysis of metals in solid materials. The first of these techniques is the direct analysis of the solid material and the second one is the liberation of the metals either by decomposition of the matrix or extraction of the metal from the matrix using appropriate solvents. The latter being the most common. The samples can then be analyzed for the presence of metals by using various analytical techniques such as Inductively Coupled Optical Emission Spectroscopy (ICP-OES) (Dean, 2003).

This section will focus and highlight the methods employed in the decomposition of the matrix in order to liberate the metals. This is because the method to be employed in this research work involves an aqua regia digestion of the sewage sludge and so a closer look and a discussion of some of the techniques commonly used is fitting.

1.5.1.1 Decomposition Techniques

In the decomposition of solid samples, the analyte (metal) of interest is liberated from an interfering matrix through the use of reagents like mineral/oxidizing acids or fusion flux and/or heat. However the use of mineral acids as reagents and external heat sources can cause problems. Some of these problems in elemental analysis include the risk of potential contamination of and loss of analytes (Dean, 2003).

1.5.1.2 Acid Digestion

This is a process which involves the use of mineral or oxidizing acids and heat sources to achieve the complete dissolution of the analytes as well as the complete decomposition of the sample matrix while avoiding loss or contamination of the analyte (Berghof, 1996). Selecting either an individual acid or a combination of acids solely depends on the nature of the matrix to be decomposed. A typical example illustrating this point is that of the digestion of a matrix containing silica (SiO_2) where the digestion is only achievable with the hydrofluoric acid (HF) since there is no other acid or combination of acids that can liberate the metal of interest from the silica matrix (Dean, 2003). The table below is compiled from Berghof (1996) and Dean (2003) and it lists a summary of the most common acid types used and their applications.

Table 1: The different types of acids mostly used for wet decomposition techniques

Acid	Boiling Point (^oC)	Remarks
Sulfuric (H_2SO_4)	340	- A non-oxidizing acid suitable for releasing volatiles, Dehydrates organic materials e.g. plastics - Digests plastics, ores and minerals & Must never be used in PTFE vessels
Nitric (HNO_3)	122	- Oxidizing acid $(CH_2)_n + 2HNO_3 \rightarrow 2NO + 2H_2O$ - Often mixed with H_2O_2 or HCl, HF, H_2SO_4 - Liberates trace elements as the soluble nitrate salt - Useful for dissolution of metals, alloys and biological samples
Hydrochloric (HCl)	84	- A non-oxidizing acid & a weak reducing agent - Dissolves salts of weaker acids (e.g. CO_3^{2-}, PO_4^{3-}) & Not generally used for the dissolution of organic matter
Hydrofluoric (HF)	108	-A non-oxidizing acid & Mainly used for decomposition of silicates, $SiO_2 + 6\ HF \rightarrow H_2SiF_6 + 2H_2O$ - **CAUTION**: Toxic & reacts rapidly with glass containers - Often employed in a mixture of other acids for the digestion of minerals, ores & soil.
Perchloric ($HClO_4$)	203	- Strong oxidizing agent for organic matter at fuming temperatures - **CAUTION**: Violent & explosive reactions may occur if not monitored - Usually the samples to be digested with this acid are pre-treated with HNO_3
Nitro hydrochloric	~200	- A 3:1 vol/vol mixture of HCl:HNO_3 referred to as 'aqua regia' - Forms a reactive intermediate NOCl and releases chlorine as the active component, $2NOCl \rightarrow 2NO + Cl_2$ and it is used for the digestion of soil, sewage and sludge samples

1.5.1.3 The chemistry of Acid Digestion

There is a wide variety of reagents that are used to carry out acid digestions aside from the mineral acids and some of these reagents are boric acid and many more. Similarly as mentioned before, the selection of the reagents to use or the preparation of the reagent mixture depends on the nature of the sample to be digested (Berghof, 1996). For organic samples, the material is generally decomposed into carbon dioxide by the use of oxidizing acids (nitric acid) in combination with reagents like hydrogen peroxide for complete mineralization. Prior to oxidative attack of plastic materials, an *in situ* dehydration of the plastic is performed by the addition of sulfuric acid. This dehydration process requires extreme caution to be taken especially when the samples are reactive i.e. materials whose decomposition process in such conditions is exothermic (Berghof, 1996).

1.5.1.4 Microwave-assisted wet digestion

Microwave digestion is a technique used by scientists for the dissolution of heavy metals in the presence of organic molecules prior to the analysis by inductively coupled plasma, atomic absorption, or atomic emission spectroscopy. This technique is achieved by exposing the sample to a strong acid (e.g. hydrofluoric acid) in a closed vessel and raising the temperature and pressure through microwave irradiation (Kingston & Jamie, 1988). The speed of thermal decomposition of the sample as well as the solubility of the heavy metals in solution are both increased by the increase in temperature and pressure of the low pH sample medium. There are several advantages of using a microwave for digestion over a conventional hot-plate. Some of these advantages include; reduction in sample loss and contamination (through the use of closed vessels), rapid and simultaneous heating of many sample solutions (typically 8 – 12). Lastly, when the set point temperature is reached the decomposition reactions continue at a similar rate as that of heated stainless steel pressure digestion vessels thereby making microwave digestions take only 20 – 40 minutes (Berghof, 1996).

1.5.1.5 Dry ashing

This type of decomposition technique is performed by placing the sample in an open inert vessel like a porcelain crucible and subjecting it to excessive heating on a muffle furnace with a temperature of up to 550 ^{o}C to destroy the combustible (organic) portion (Gaines & Brollin, 2003). This technique is deemed to be the simplest of them all and after the decomposition; the residue (ash) is dissolved in acid prior to the analysis (Dean, 2003). The Dry Ashing technique is mainly used for combustible or organic materials and has a number of advantages and disadvantages. The advantages include the ability of decomposing large sample sizes, the use of little or no chemical reagents at all and the ability of preparing the samples with volatile combustion elements such as sulfur, chlorine (Gaines & Brollin, 2003). The disadvantages associated with the dry ashing technique include; the loss of sample material due to volatilization, contamination from the muffle furnace, physical loss of 'low density' ashes due to air currents i.e. when the muffle door is opened and contamination from the ashing container (Gaines & Brollin, 2003).

1.6 Instrumentation

1.6.1 Background

The knowledge of the chemical composition of a sample and the concentrations of the elements present within are of interest to analysts. There are only 92 naturally occurring elements and millions of different molecules and this makes it an easy task to differentiate among elements than molecules. Nonetheless, the composition of the elements in a sample is often an important part of the information required for the assessment of its properties. Most analytes in samples are available at trace level concentrations and it is for this reason that sensitive analytical methods & instrumentation for trace level measurements are required (Boss & Fredeen, 1997).

The most commonly used techniques for the determination and quantitation of trace concentrations of metals and metalloids in environmental samples are based on atomic spectrometry. These techniques involve electromagnetic radiation or light that either gets absorbed by or emitted from the atoms of the sample. The amount of electromagnetic radiation that is either absorbed or emitted by the atoms of the sample can be related to the quantitative information (concentration) and the qualitative information (e.g. the elements present) is related to the wavelengths at which the radiation is absorbed or emitted. Another technique associated with atomic absorption or emission spectrometry is atomic mass spectrometry. In this technique, the ions introduced into a mass spectrometer are separated according to their mass to charge (m/z) ratio and they can either be detected qualitatively or quantitatively. Therefore, through using atomic spectrometry techniques a great deal of meaningful quantitative and qualitative information about a sample can be gathered (Boss & Fredeen, 1997).

1.6.2 Atomic spectrometry

In atomic spectrometry techniques mostly used for the analysis of trace elements, intense heat is used for decomposing the sample into a cloud of hot gases comprising of free atoms and ions of the element that is investigated. There are various types of atomic spectroscopic systems (see figure 7) and the arrangement of each of these differs from the others. However what is common to all is that there must be a source of light, a flame or plasma, a monochromator or a mass spectrometer in the case of atomic mass spectrometry and lastly, a detector (Boss & Fredeen, 1997).

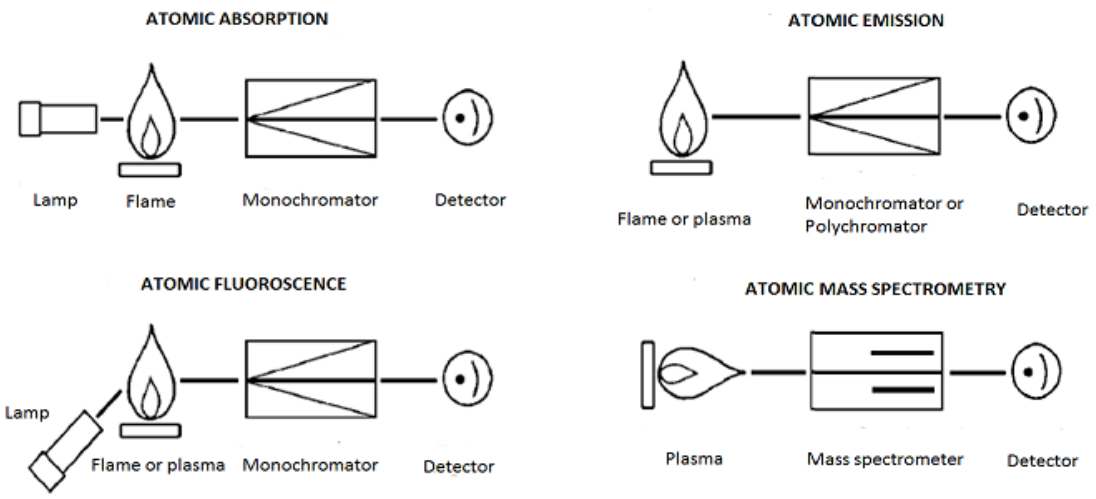


Figure 7: The different systems of atomic spectroscopy (Boss & Fredeen, 1997)

Atomic spectroscopy can be used for the determination of elemental composition of analytes by their electromagnetic or mass spectrum. There are several techniques available for performing analytical determinations and the selection of the most appropriate one is the key to obtaining accurate and reliable results. To properly select the most appropriate technique to use, one must have a basic understanding of each of the techniques since they each have their own individual strengths and limitations (PerkinElmer, 2009).

1.6.3 Flame Atomic Absorption Spectroscopy (FLAAS)

In atomic absorption spectrometry the absorption occurs when the atom in the ground state absorbs energy in the form of light of a specific wavelength and gets promoted to an excited state. The increase of the number of atoms of the selected element in the light path occurs due to the increase of the amount of light energy absorbed at a specific wavelength. The amount of light that is absorbed by the atoms can be related to the concentration of analytes present in known standards to determine the concentrations of unknown samples through measuring the amount of light they absorb. Conducting an experiment on an atomic absorption spectroscopy requires a primary light source; a source of atoms; a monochromator for the isolation of a specific wavelength of light to be measured; a detector for an accurate measurement of light and electronics for processing the data signal and a data display system (e.g. software) to show the results (PerkinElmer, 2009). Table 2 summarizes the strengths, limitations and applications of these different instruments. The light source used is a hollow cathode lamp (HCL) or an electrode-less discharge lamp (EDL). In general for every element to be determined, a new lamp is used and by new lamp this means for example a Cd lamp for the determination of cadmium. However, there are cases where the

combination of the elements is in one lamp. Modern instruments use solid-state detectors instead of photomultiplier tubes that were previously used. To produce free-atoms the source of energy used is heat and it comes in the form of an air/acetylene or nitrous-oxide/acetylene flame and the sample is then introduced as an aerosol into the flame by the nebulizer and spray chamber. Lastly, the light gets absorbed due to the alignment of the burner head to allow the passage of the light beam through the flame (PerkinElmer, 2009

1.6.4 Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)

In Graphite Furnace Atomic Absorption (GFAA), the liquid sample is placed on a small cup in the furnace and then directly introduced into the graphite tube which is then heated in a series of programmed steps to remove the solvent, pyrolyze and finally atomize the remaining sample. The matrix modifier is continually added to the sample before heating in order to control the chemical form of the analyte during the heating sequence. The analyte gets completely atomized and these atoms are retained within the tube for a longer period of time and in this way the sensitivity and detection limits are improved greatly improved over Flame Atomic Absorption (PerkinElmer, 2009).

1.6.6 Inductively Coupled Plasma Mass Spectroscopy (ICP – MS)

Inductively Coupled Plasma-mass Spectrometry (ICP-MS) incorporates the ionization efficiency of the argon plasma with the sensitivity and selectivity of mass spectrometric separation (Garbarino & Taylor 1996). The singly charged analyte ions produced in the plasma are directed into the mass spectrometer and separated according to their mass-to-charge ratio. Only ions of a selected mass-to-charge ratio are directed to a detector that determines their presence. This detector is mounted 90 degrees off-axis in order to reduce the background signal contributions from the photons emitted by the plasma (Garbarino & Taylor 1996). The quadrupole mass spectrometer is often used because of its robustness, speed & ease of use and due to the similarities of sample introduction and data processing techniques the ICP – MS is very much similar to the ICP – OES system (PerkinElmer, 2009).

Table 2: The summary of the strengths and limitations of the instruments for chemical analysis

Instrumental Technique	Strengths	Limitations	Applications
Flame AA - Atomic Absorption Spectroscopy	- Relatively easy to use - Wide application information available - Relatively cheaper	- Suffers from low sensitivity and chemical interferences. - Analyses one element at a time - Flammable gas is a potential hazard; therefore it can't be left unattended.	- Suitable for laboratory analysis of large numbers of samples for a restricted number of elements. - Ideal for the determination of major components and analytes of a higher concentration.
GFAAS - Graphite Furnace Atomic Absorption Spectroscopy	- Very low detection limits - Automation - Large number of applications	- Analytical working range is limited - The sample throughput is often less than in other techniques	This technique is suitable for the laboratory scale analysis of a limited number of elements requiring very low and good limits of detection.
ICP-OES - Inductively Coupled Plasma Optical Emission Spectroscopy	- Best multi-element atomic spectroscopy technique - Good limits of detection - Easy to use - Broad analytical range - Automation	- Highly cost effective - Spectral interferences	Suitable for the analysis of a large number of elements in moderate or large samples.
ICP-MS - Inductively Coupled Plasma Mass Spectrometry	- Can perform isotopic analysis - Multi-element capabilities - Detection limits are consistently in the 0.1 µg/L range. - Automation	- Highly cost effective - Method development is difficult	Suitable for the laboratory analysis of multiple elements in a large number of samples.

1.6.5 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

In this technique, the sample is subjected to high temperatures that allow its dissociation into atoms which further undergo collisional excitation and ionization. The atoms or ions in the excited states can either decay to lower energy states through thermal and/or emission energy transitions, hence the name 'emission' spectroscopy. The intensity of the light emitted at a specific wavelength for each element is measured and used to find the concentration of the element (PerkinElmer, 2009).

The greatest advantage of the ICP-OES comes from the excitation properties of the high temperature sources it uses and these thermal excitation sources can inhabit different energy levels for different elements all at the same time. The atoms and ions in their excited state can then emit their characteristic radiation at different intervals thus providing a flexibility to choose from several different emission wavelengths for an element. Furthermore, this also results in the ability to measure the emissions produced from several different elements simultaneously. This feature, however, also has a disadvantage associated with it and it is that as the number of emission wavelengths increases, the probability for the interferences from emission lines that are too close in wavelength also increases (PerkinElmer, 2009).

1.6.6 Vapour Generation Techniques

In chemical vapor generation the analyte is separated from the sample matrix by the generation of gaseous species as a result of a chemical reaction. This technique has received its broad application in atomic absorption spectrometry in the forms of cold vapour AAS for the determination of mercury and hydride generation AAS for elements that form gaseous hydrides. Chemical vapour generation is also used together with optical emission and atomic fluorescence spectrometry (Inczédy *et al*., 1997).

The generation of carbonyls, halogens and other volatile species have so far only been used rather remarkably in spectrochemical analysis. The generated gaseous species is introduced with a stream of purge gas into a sampling source for measurement by atomic absorption spectroscopy or other atomic spectrometric methods. The vapour-generating assembly, including gas flow controls and means for the addition of reactants used for the production of the volatile species, is the hydride generator, mercury-vapor generator or other generator (Inczédy *et al*., 1997).

In batch mode generation, specified volumes of the sample and reactant solutions are mixed at the beginning of the measuring process. The formed volatile species are liberated in the generator vessel and are accelerated by stirring, agitating or by passing a purge gas through the solution (Inczédy *et al*., 1997). In continuous mode, the blank or sample solutions are added successively to a continuous flow of the reactant. The volatile species formed are liberated from the reacting solution spontaneously in a phase separator (Inczédy *et al*., 1997).

The mercury vapour generator is of interesting pertaining to this current research as it was the tool used for the determination of mercury in the sewage sludge. Mercury has a relatively poor sensitivity in traditional flame atomic absorption and as a result, alternative atomization techniques for AA determination of this metal have been developed. Furnace methods for mercury determination are not recommended due to its extreme volatility which has a significant vapor pressure even at room temperature. The cold vapour atomic absorption technique for mercury has received the greatest attention since its first proposal by Poluektov and co-workers in 1963 (Dominski & Shrader, 2010). The actual operation and the principles of the vapor generation technique are discussed fully in the methodology section.

1.7 Using standardized procedures for quality control and improving accuracy

1.7.1 Quality assurance and Standard Operating Procedures

Quality assurance refers to the full range of practices used in ensuring that laboratory results are reliable and it is defined as the system of documenting and cross referencing the management procedures of the laboratory (Briggs *et al.,* 1996). The objective of quality assurance is to ensure that clear and concise records of all procedures which may have a bearing on the quality of data in order for those procedures to be monitored with a view ensuring that quality is maintained (Briggs *et al.,* 1996).

There are several components of quality assurance and these are management, training, laboratory facilities, equipment maintenance and calibration, sampling, sample receipt, storage and disposal, reporting of results (Briggs *et al.,* 1996). One other component is "Standard Operating Procedures" and this is discussed separately because a standard operating procedure (e.g. ISO 11466) was used for this research. Standard Operating Procedures provide the core of most of the day to day running of any quality assurance programme. They are the documents describing in detail every procedure conducted by the laboratory. This includes sampling, transportation, analysis, use of equipment & the production of reports (Briggs *et al.,* 1996).

1.7.2 Internal Quality Control

Internal quality control consists of the operational techniques used at the laboratory for continuous assessment of the quality of the results of individual analytical procedures (Briggs *et al.,* 1996). The main focus is on monitoring precision as well as accuracy. "Internal quality control is part of the wider quality assurance programme, but differs from it by virtue of the emphasis placed on quantifying precision and accuracy" (Briggs *et al.,* 1996).

1.7.3 External Quality Control

External quality control (EQC) is the means of establishing the accuracy of analytical methods and procedures by comparing the results of analyses made in one laboratory with the results obtained by other laboratories performing the same analysis on the same material (Briggs *et al.,* 1996). This is usually accomplished by one laboratory (i.e. the reference laboratory) sending out sets of specimens with known and unknown concentrations of the tested variables to all of the participating laboratories (Briggs *et al.,* 1996).

Each participant analyses the specimens for the specified variables and sends reports to the reference laboratory. All the results from the participating laboratories are ordered by the organizers of the External Quality Control program and then subjected to detailed statistical analysis (Briggs *et al.,* 1996). Thereafter a report is generated for each laboratory, giving a target value for the reference samples as well as a histogram illustrating distribution of results for each material and lastly, an individual performance score relating the individual laboratory results to the target value (Briggs *et al.,* 1996).

The calculations for performance indicators are quite complex because a lot of specimens have to be considered and the method variance varies with the concentration of the variable. However, the general principle of providing a method of performance comparison remains the same in all EQC exercises (Briggs *et al.,* 1996).

1.8 Aims of the project

Umgeni Darvill Waste Water Works (UDWWW) utilizes the ISO 11466 method in the determination of metal concentration in their sewage sludge. This standard method involves the extraction of trace elements from soils and other similar materials (e.g. sewage sludges) through digestion with the aqua regia. The sample preparation method to be used for this work entails grinding the samples prior to acid digestion in order to achieve greater sample homogeneity while increasing the efficiency of acid attack. Ultimately, the acidic solutions resulting from the aqua regia digestion are then prepared and made suitable for atomic spectroscopic analysis.

***The main aims of this research were as follows*:**

1. Using the cause and effect (Ishikawa) diagrams to identify the main sources of errors in the determination of the concentrations of metals in the sewage sludge.
2. Quantifying & combining the uncertainty components from the Ishikawa diagrams in order to calculate the overall uncertainty in the concentrations of the metals.

***The objectives set at the beginning of this work were the following*:**

1. Defining a set of operating procedures that will lead to accurate, reliable and robust instrumental methods for the determination of metals in the sewage sludge samples with well-defined and appropriate levels of uncertainty.
2. These operating procedures will include using the different sample preparation techniques i.e. different sampling strategies in order to assess for an effect in the errors of the measured concentrations.
3. Optimizing methods for the atomic absorption instrumentation to be used, e.g. Graphite Furnace AA, for the analysis of cadmium in the sewage sludge as well as operating methods for the analysis of arsenic, lead and chromium in the ICP-OES.
4. Using the vapour generating technique coupled with the AA and/or ICP-OES for the analysis of mercury in the sewage sludge.

CHAPTER 2

2. Optimization & Methodology

2.1 Determination of moisture and solids content

The sewage sludge samples were dried overnight for 6 hours in an oven set at 100 ^{o}C. On the day following the overnight drying, these samples were allowed to cool in a desiccator with silica gel as a drying agent before re-weighing. From there, the moisture & solids content of the samples was calculated and expressed as a percentage of the dried mass, see appendix A for the calculations.

2.2 Sampling Techniques

2.2.1 Digestion of sieved and ground sewage sludge

Coning and Quartering

A total mass of 50 g of the oven dried sewage sludge was sieved using a sieve mesh (2.00 mm aperture, ISO 3310 – 1). The sieved sewage sludge was transferred into a mortar and finely ground with a pestle in order to improve the reproducibility of the analytes to be measured. Consequently, the efficiency of acid attack increases upon grinding due to the increase of the surface area of the particles (ISO, 1995). Once ground, the sludge was then coned and quartered into four subsamples and from each of these subsamples, 3 g was accurately weighed into four decimal places using the analytical mass balance. The digestion of the subsamples was performed using aqua regia and the concentrations of the arsenic, cadmium, chromium and lead were analysed with the ICP – OES.

Random sampling

The sewage sludge used for this random sampling was the same 50 g used in coning & quartering above. After coning & quartering was performed, the remaining sewage sludge was transferred back to the plastic bag and given a thorough shake to mix the contents. The four 3 g subsamples were randomly taken directly from the plastic bag and no special technique was used. These subsamples were accurately weighed to four decimal places and digested with aqua regia and then taken for metal concentration determination in the ICP – OES.

Non-sieved and non-ground sewage sludge samples

For this subsampling technique, the four 3 g subsamples were taken directly from the plastic bag and they were neither sieved nor ground. These subsamples were weighed to four decimal places and digested with aqua regia and then taken for metal concentration determination in the ICP – OES.

2.3 The acid digestion method (ISO 11466)

The procedure or guideline used at Umgeni Darvill for the digestion of their soil or sewage sludge samples is ISO 11466:1995 (E) for Soil quality — Extraction of trace elements soluble in aqua regia. This guideline is summarized below and also on a schematic diagram in figure 8. For each sewage sludge subsample that was digested, approximately 3.0 g of it was accurately weighed on a four decimal place mass balance and carefully transferred into a 250 ml conical flask used as a reaction vessel. The sludge was then moistened with 1.0 ml of distilled water followed by the addition of 21 ml of 32% (v/v) hydrochloric acid and 7 ml of a 65% (v/v) nitric acid. The volumes of these two acids were in a 3:1 ratio. The nitric acid was added dropwise in order to reduce foaming.

The resulting mixture was left standing at room temperature for 16 hours without heating. After this period, complete extraction was attained and this led to allowing the reaction mixture to subside and then followed by the addition of an extra 1.0 ml of HNO_3 to rinse the sides of reaction vessel. The mixture was refluxed at 120 ^{o}C for a period of 2 hours and after refluxing, the sediment free supernatant was filtered through a Grade 1 70 mm Whatman filter paper into a 100 ml volumetric flask. The filter paper was rinsed with no more than 3.0 ml of HNO_3 and the volume was made up to the mark with a freshly prepared 0.5 mol. L^{-1} HNO_3 solution.

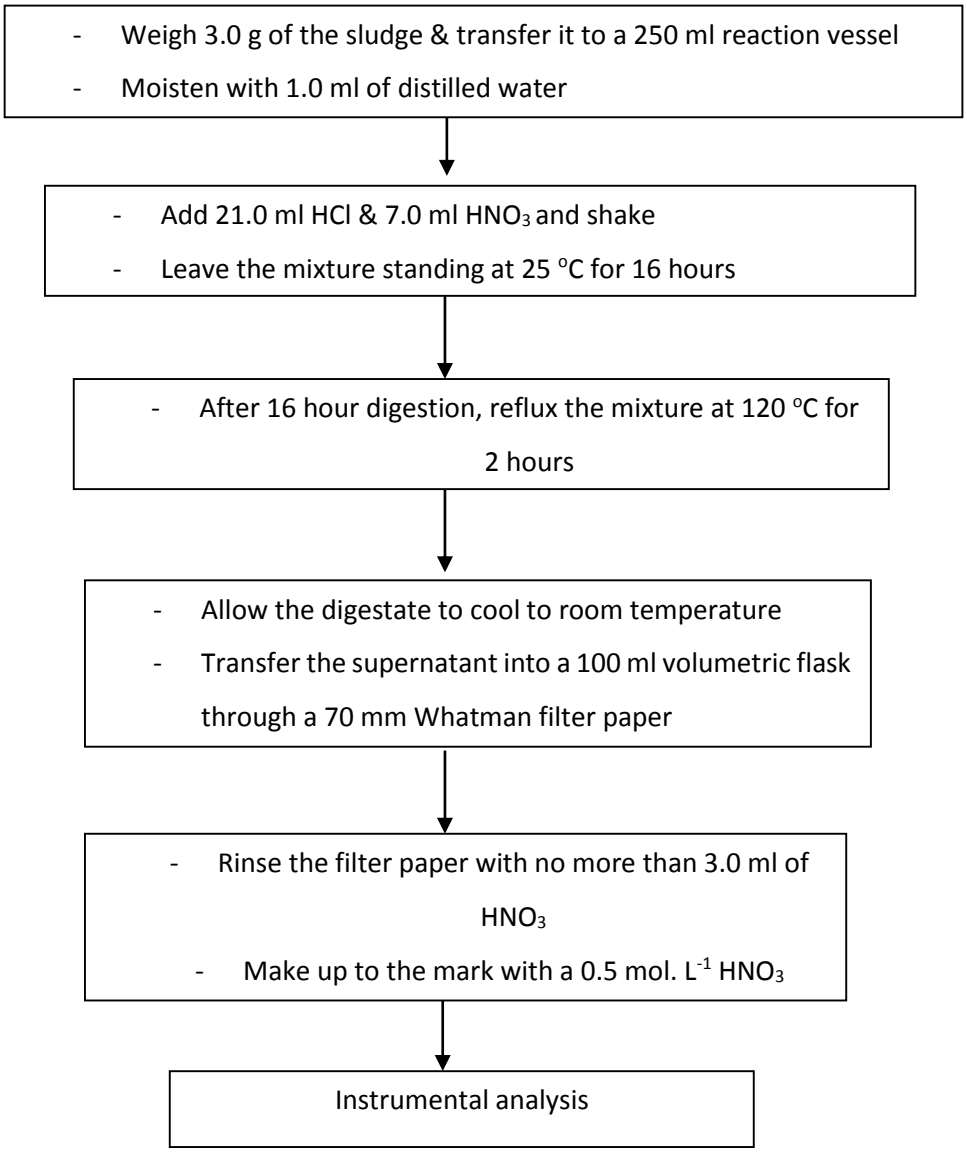
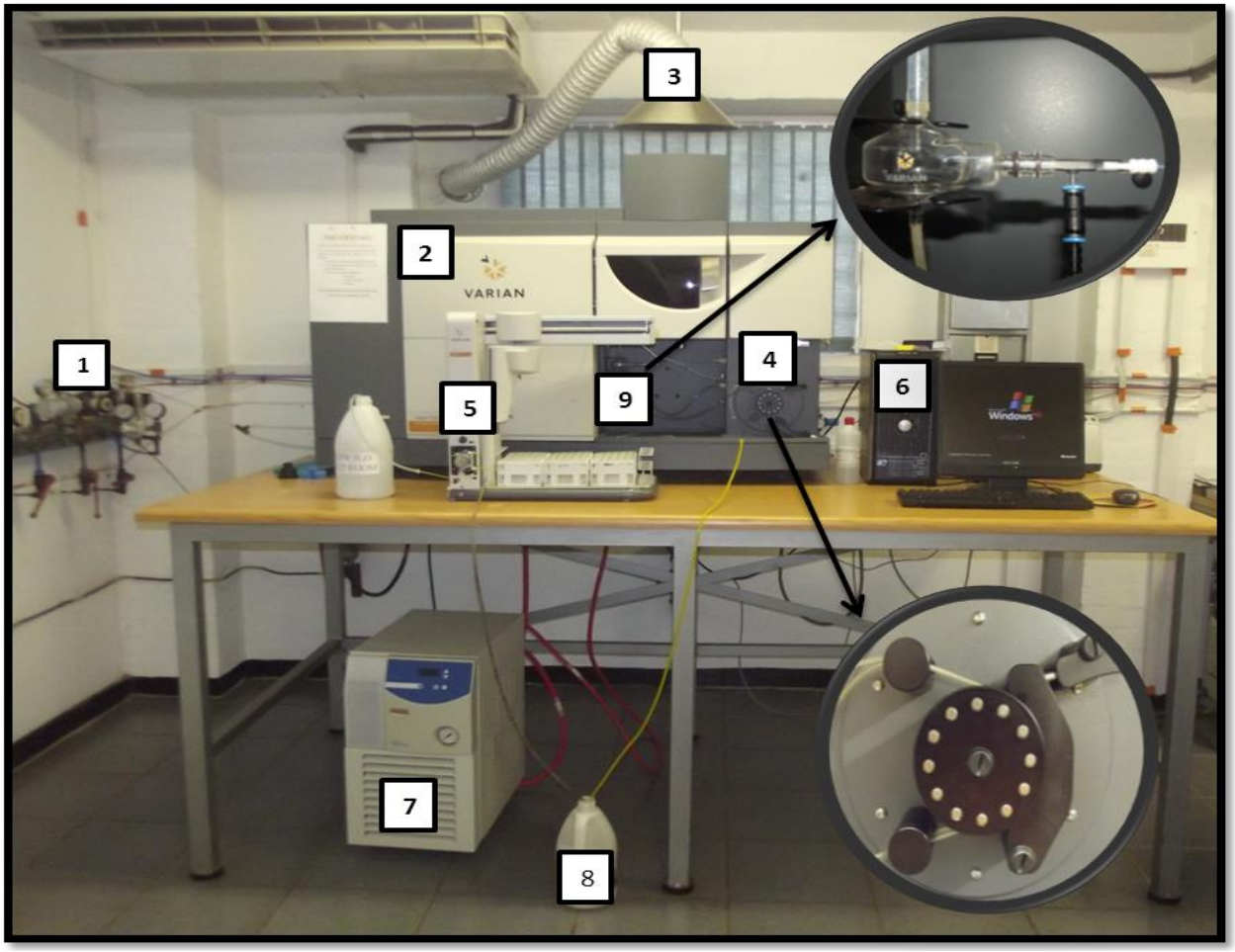


Figure 8: The acid digestion of the sewage sludge samples (ISO 11466)

2.4 Optimization and method development for the ICP-OES

2.4.1 The ICP-OES used for this research

There are various types of ICP-OES instruments that are commercially available and all of them have similar components and carry the same function. The one that was used for this current research was the VARIAN 720-ES ICP-OES shown in figure 9 below.



1 – Argon gas tap, 2 – ICP-OES instrument, 3 – Extractor fan, 4 – Peristaltic pump, 5 – Autosampler, 6 – Computer, 7 – Cooling tower, 8 – Waste container, 9 – Nebulizer & Spray Chamber

Figure 9: The VARIAN 720-ES ICP-OES instrument used for this research

As mentioned previously, the liquid sample is introduced to the plasma in the form of an aerosol through the nebulizer. This step is very crucial since only small droplets of the sample are used. A variety of forces can be used for breaking up a liquid sample into an aerosol but only pneumatic forces and ultrasonic mechanical forces have shown success when used with an ICP (Boss & Fredeen, 1997). A large number of commercial ICP nebulizers are of the pneumatic type and these use high speed gas flows for the creation of an aerosol. For this research, the VARIAN 720-ES ICP-OES, made use of a concentric nebulizer. The solution is pumped from the peristaltic pump through a capillary tube to the nebulizer where it gets transferred to a low-pressure region formed by a gas flowing past the end of the capillary tube. The solution is then broken up into an aerosol by the low pressure and high speed gas combination. After the aerosol has been created by the nebulizer, it is then transported to the torch for injection to the plasma. The spray chamber is designed to remove large droplets from aerosol and it is placed between the torch and the nebulizer in order to form a spray of very small droplets which are carried by an inert gas (argon) for injection into the plasma (Boss & Fredeen, 1997).

The light that is emitted by the excited species in the plasma is measured in order to obtain the information about the sample. Since these excited species in the plasma emit light at several different wavelengths, the emission obtained from the plasma is said to be polychromatic. This polychromatic radiation is partitioned into individual wavelengths in order to allow the identification of the emission from each excited species and the intensity of this radiation is measured without interference from emission at other wavelengths. The separation of light according to wavelengths is achieved using either a monochromator which measures light one wavelength at a time or a polychromator which measures light at numerous different wavelengths at once. The detection of light after separation from other wavelengths is achieved through the use of a charge-coupled device (CCD) (Boss & Fredeen, 1997).

After detecting the light from the wavelengths, the step that follows is to extract qualitative and quantitative information about the sample. To know what elements are present in the sample, i.e. obtaining qualitative information, involves identifying the presence of emission at the wavelengths ascribed to the elements of interest. To classify whether an observed emission line belongs to the element of interest, at least three spectral lines are examined. However, the possibility of spectral interferences from other elements may bring uncertainty about the presence of the element of interest in the plasma but nonetheless this may be overcome by choosing between several different emission lines for the element of interest since a relatively large number of them is available.

Moreover, obtaining quantitative information is accomplished by plotting the emission intensity against the concentration, i.e. the calibration curves. The solutions with known concentration of the elements of interests i.e. the standard solutions are introduced into the ICP instrument and the intensity of the emission for each element or analyte is measured (Boss & Fredeen, 1997).

2.4.2 The operating conditions and specifications of the VARIAN 720-ES ICP-OES

The operating conditions and specifications of the VARIAN 720-ES ICP-OES used in this research are shown below. The analytes chosen for this wok were arsenic, cadmium, chromium and lead. The selection of the wavelengths used for the determination of these analytes is also discussed in the sections 2.4.2 to 2.4.3.

ICP characteristics

Manufacturer and Model	VARIAN 720-ES ICP-OES
Frequency	40 MHz
Power	1.00 kW

Conditions set

Plasma argon flow rate (L/min)	15.0
Auxiliary argon flow rate (L/min)	15.0
Nebulizer flow (L/min)	0.75
Instrument stabilization time (s)	15
Replication time (s)	1
Pump rate (rpm)	15
Rinse time (s)	10
Sample uptake delay time (s)	1
Replicates	3

2.4.3 Selection of the appropriate wavelengths for each of the elements

Arsenic (As)

Arsenic had two potential wavelengths to use for its analysis on the ICP-OES and these are shown in the table 3. These two wavelengths had the highest intensities of all thus making them eligible candidates for measuring the arsenic. However, the arsenic concentrations measured for the digested sewage sludge 'test samples' were highly reproducible and consistent for the 188.90 nm line and that made this wavelength to be favourable.

Table 3: The different wavelengths of the arsenic and their respective intensities

Species	Wavelength (nm)	Intensity
As	188.980	531.6
	193.696	384.5

Moreover, the majority of the arsenic determination studies using the ICP-OES have used 188.980 nm as the optimum and highly sensitive wavelength. An example of such a study is that which was conducted by Antin *et al.* (2010) at the National Measurement Institute for the 'proficiency study AQA 101 metals in soil'. In that study there were very low detection and quantitation limits reported for the method. Moreover, the recovery percentage of the spike solution was also very high thus demonstrating the robustness of their method.

Cadmium (Cd)

The cadmium element had a pool of wavelengths to choose from and all the different lines had very high intensities which made them possible candidates, see table 4. Contrary to the other elements, cadmium had (I) and (II) which represented ionic or atomic lines due to the Zeeman Effect and these had high emission intensities.

Table 4: The different wavelengths of the cadmium species and their respective intensities

Species	Wavelength (nm)	Intensity
Cd (II)	214.439	30469.0
	226.502	38378.5
Cd (I)	228.802	1362.0
	508.582	1785.9

As high as the intensities are for the Cd (I), the concentrations of the cadmium measured for the digested sewage sludge 'test samples' were irreproducible and inconsistent. This meant that these two wavelengths were not eligible to be used. From the two remaining Cd (II) wavelengths, the 226.502 nm line was shown to have the highest reproducibility of the cadmium concentrations although the presence of interference was suspected, see later in results and discussion.

Chromium (Cr)

The wavelength chosen for the chromium was 267.716 nm (see table 5) as it had the highest intensity compared to the other wavelengths which were not used due to the interferences observed from other elements.

Table 5: The intensity of the wavelength line chosen for Chromium

Species	Wavelength (nm)	Intensity
Cr	267.716	33439.0

A study conducted by Chochorek *et al.* (2010) for the ICP-OES determination of select metals in surface water showed that a wavelength of 267.716 nm for chromium had a higher precision (expressed as the relative standard deviation). Although metals in surface water are not like metals in the sewage sludge as it is the case in this current research but the principle of wavelength selection was the same as that of the study by Chochorek *et al.* (2010). Moreover, Thompson & Walsh (1983) recommend the line at 267.654 nm for the analysis of chromium in soil and the intensity at this wavelength is similar to that of the 267.716 nm chosen for this research.

Lead (Pb)

The highest intensity for the lead was observed at a wavelength of 220.353 nm. At this wavelength there were only few interferences observed from other metal ions and these were regarded to have no significant effect in the determination of the concentration of this element due to their low intensities, see table 6. This led into selecting this wavelength as the optimum one to use for the analysis of this element. To support the selection of this wavelength, a study conducted by the GBC Scientific Equipment Company to investigate the dependence of the various operating parameters on the wavelength demonstrated that the wavelength of 220.353 nm was the most optimum for this element (GBC, 2005).

Moreover, the study also showed that the intensity of the wavelength of the lead increased at lower flow rates and this was because the flow rate directly controls the sample transit time through the plasma thus affecting the level of atomization attained. The low nebulizer flow rates increases the residence time of the analyte in the plasma therefore giving it longer to acquire the energy necessary for high energy transitions (GBC, 2005).

Table 6: The wavelengths and intensities of the potential interferences of the lead

Species	Wavelength (nm)	Intensity
Fe	220.352	0.1
Pb	220.353	4051.6
Mn	220.354	0.3

Table 7 below shows the list of the wavelengths that were used for the determination of each of the metal pollutants in the ICP – OES.

Table 7: The wavelengths to be used for each of the metal pollutants in the ICP-OES

Element	Wavelength (nm)
As	188.980
Cd	226.502
Cr	267.716
Pb	220.353

2.4.4 The calibration curves for the analytes

The standard solutions for calibrating the ICP – OES were purchased from Merck Chemicals and the concentration of each analyte was 1000 mg. L^{-1}. The mixed standard solutions of lower concentrations were prepared by diluting the stock analyte solutions with ultra-pure water to achieve the desired final concentrations of each analyte as shown in table 8. For example, to prepare a mixed "standard 1" with 1.00 mg. L^{-1} of As, 0.50 mg. L^{-1} of Pb, 0.20 mg. L^{-1} of Cr and 1.00 mg. L^{-1} of Cd, the volumes measured were 100 µl, 50 µl, 20 µl and 100 µl respectively. These volumes were transferred into 100 ml volumetric flasks and made up to the mark with ultra-pure water. The standards were made up with ultrapure water to eliminate the possibility of contamination or interference by dissolved metals in tap or distilled water. The standards were not matrix matched for the same amount of nitric acid as the samples. Later it will be demonstrated that the amount of nitric has no effect in the calibration standard solutions.

Table 8: The concentrations of the analytes in the standards used for calibrating the ICP-OES

Standard	Concentration of the analyte (ppm)			
	As	Cd	Cr	Pb
1	1.00	1.00	0.20	0.50
2	2.00	2.00	0.40	1.00
3	3.00	3.00	0.60	1.50
4	4.00	4.00	0.80	1.75
5	5.00	5.00	1.00	2.00

The standard solutions were measured in the ICP-OES according to the settings and instruments conditions listed already. These solutions also included a blank solution which was the ultra-pure water used to prepare the standard solutions. The instrument was set up to run the calibration once at the beginning of every analysis in order to ensure that there are no alterations on the working conditions of the instrument (e.g. the cleanliness of the torch) due to prolonged use. The calibration graphs for all of the elements are shown in the figures 10 to 13 and were all constructed using the Microsoft Excel 2010 software.

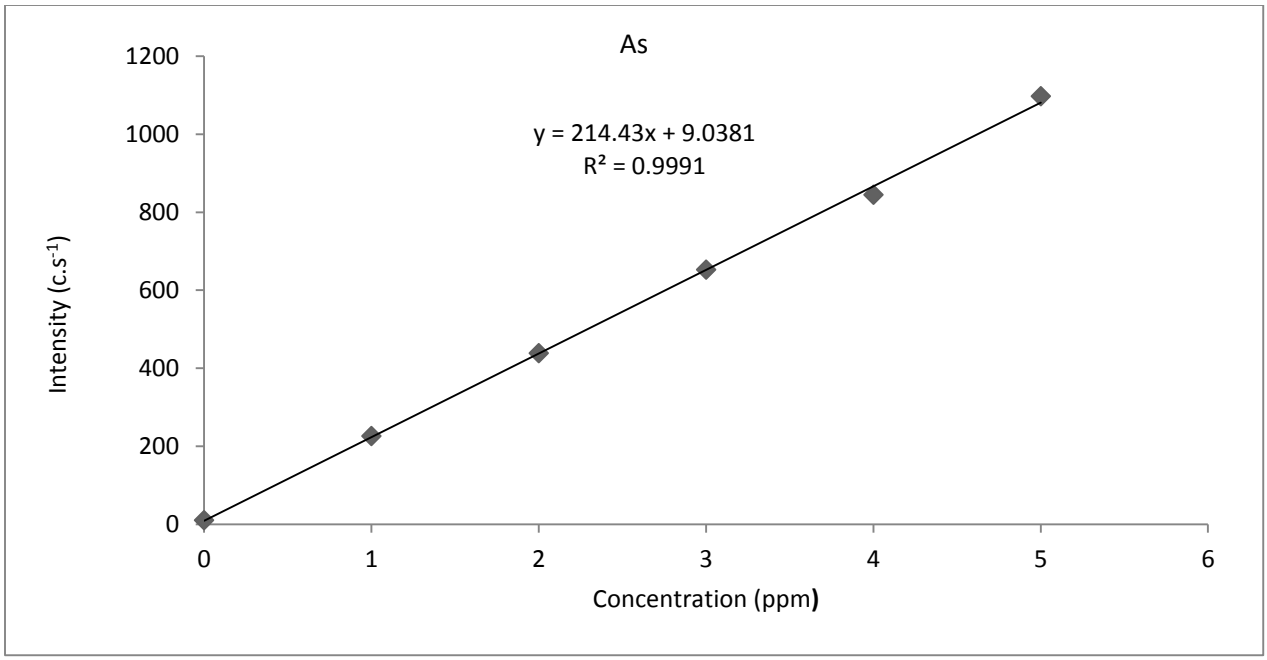


Figure 10: The calibration curve for the arsenic

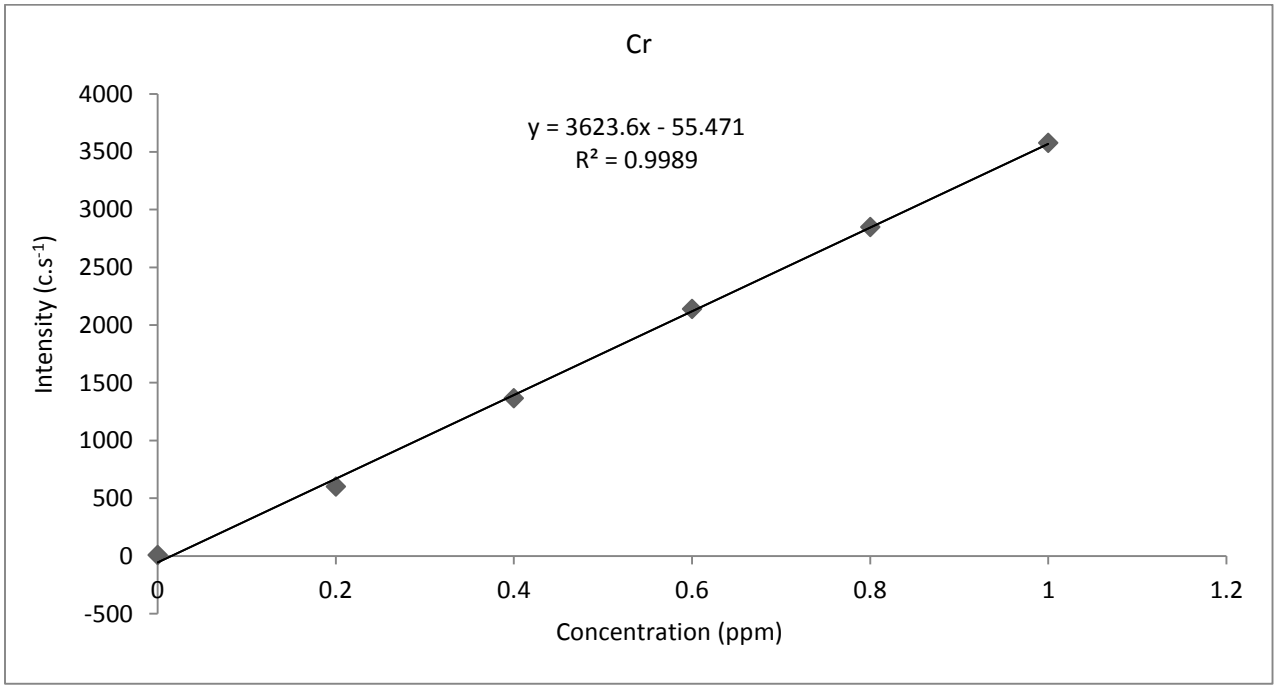


Figure 11: The calibration curve for the chromium

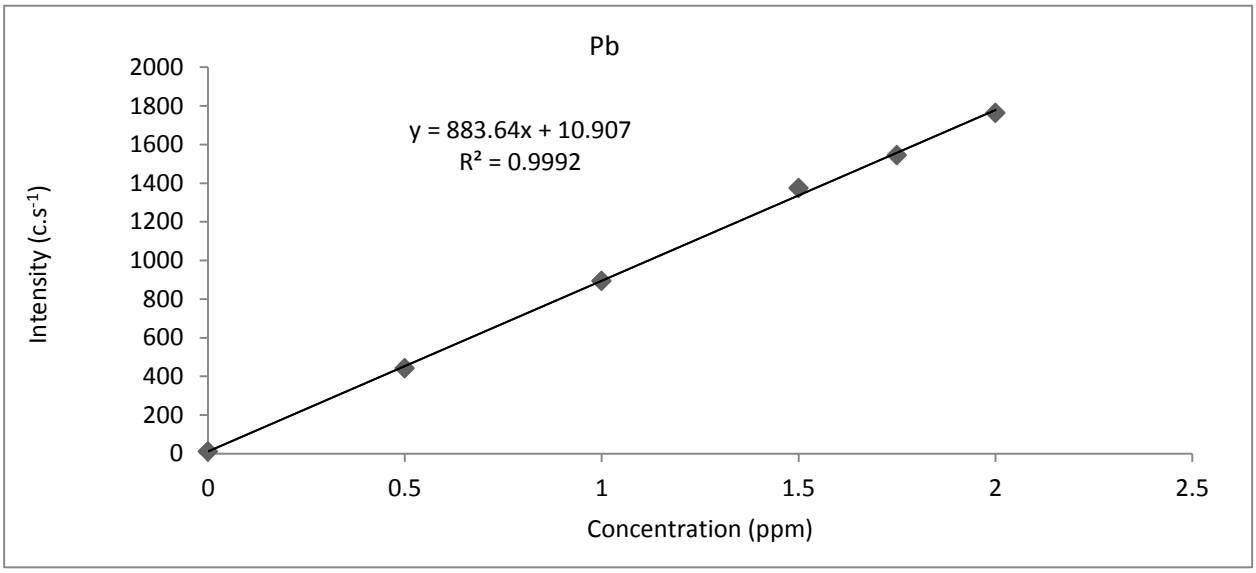


Figure 12: The calibration curve for the lead

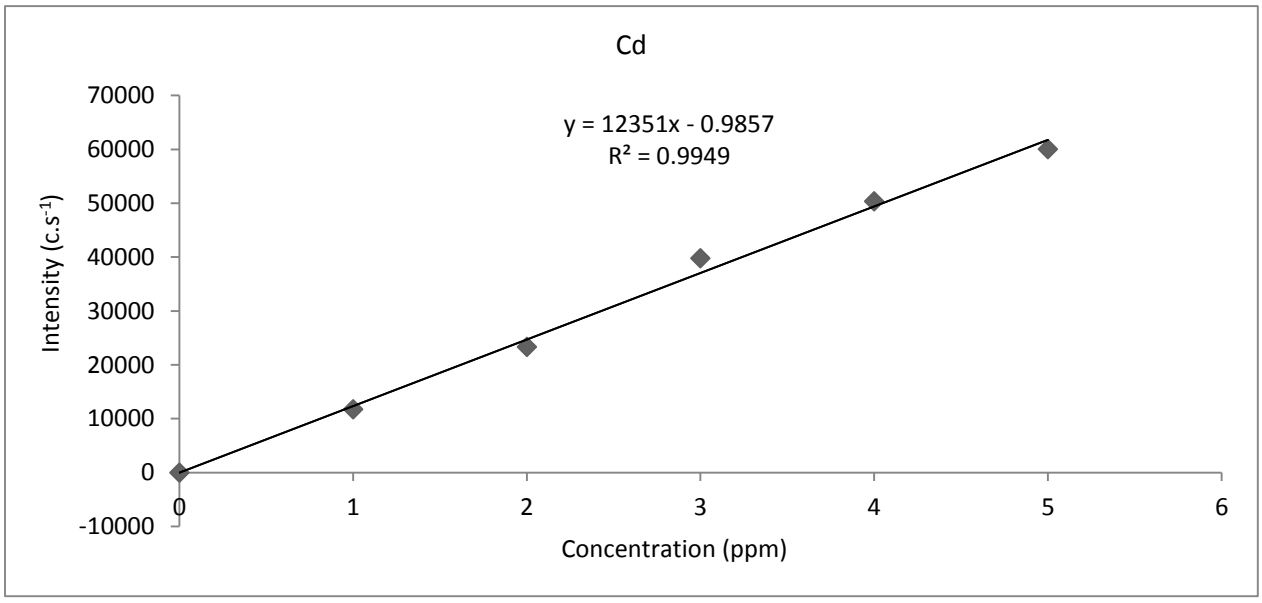


Figure 13: The calibration curve for the cadmium

2.5 The principles, optimization and method development for the GFAAS

2.5.1 The principles of the GFAAS

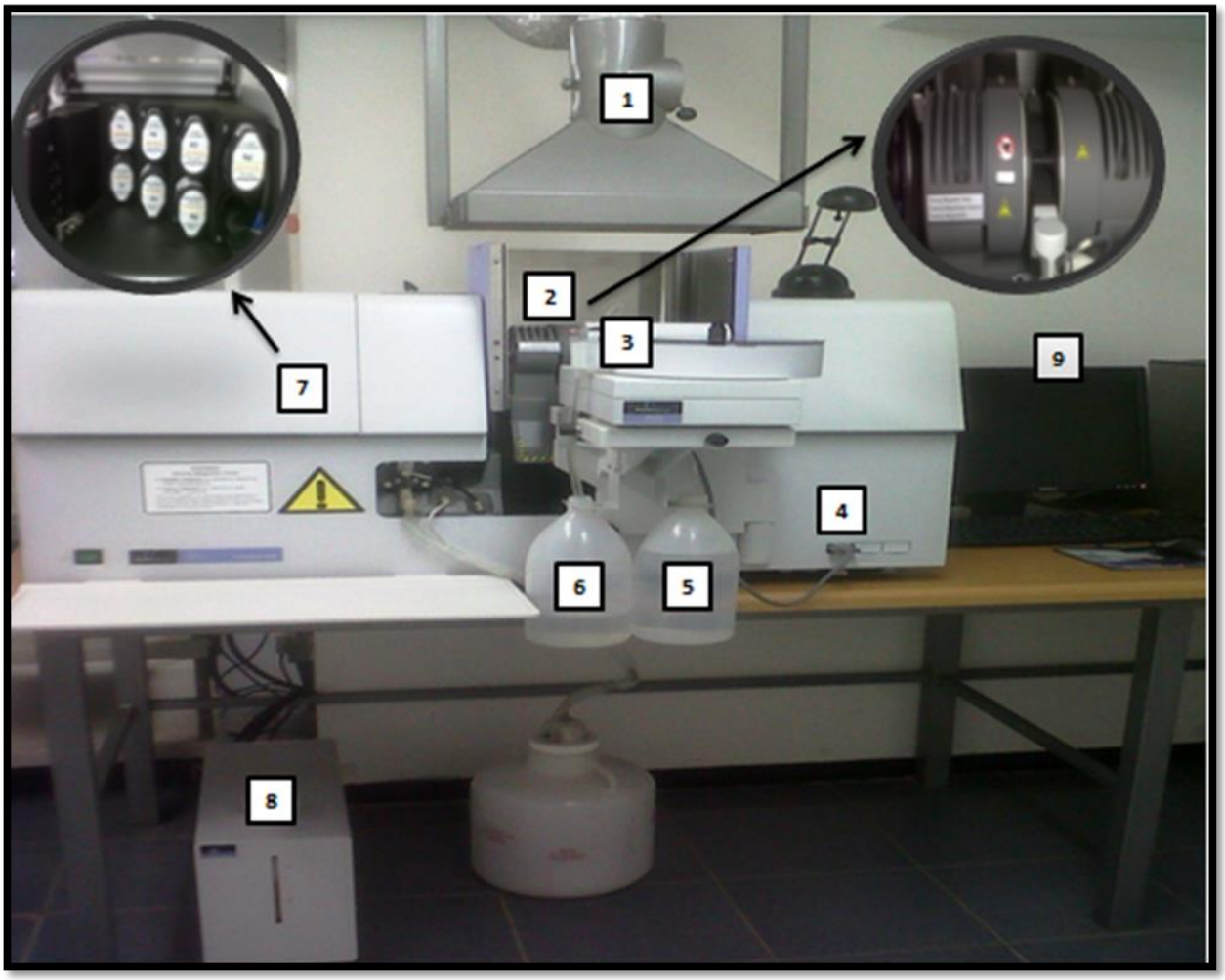
In Graphite Furnace Atomic Absorption, a known microliter volume of a sample is pipetted into a small graphite tube (the furnace) which is then heated electrically. There are three heating steps that are applied to the sample contained in the graphite furnace. These steps are the 'drying stage' which is specially designed for the evaporation of the solvent from the sample without splattering it or compromising its integrity. The second stage is 'ashing' where the temperature of the furnace is increased usually in the range of 400 – 800 ^{o}C for a specified period of time. During ashing, the organic components that may be present in the sample matrix are charred to change their chemical form and to also remove the high boiling volatile components. The importance of this stage is to simplify the sample matrix in order to reduce interferences and to also make the calibration easier. Finally, the third and last heating step is the 'atomization' stage where the temperature of the furnace is rapidly raised to as high as 2700 ^{o}C to volatilize the remaining components.

The GFAAS instrument comprises of five main components; the first one is the light source which provides bright, stable and narrow line spectra of the elements to be determined. Hollow cathode lamps made from the elements to be emitted are often used. Electrodeless Discharge Lamps are also available and provide better sensitivities for some elements. For this research, the THGA graphite furnace used patented PerkinElmer cable less Lumina lamps which are easy to connect as one simply slides them in and they are then recognized by the instrument.

The second component of the GFAAS is the atomization chamber which is a graphite tube that is electrically heated to vaporize and atomize the sample. The monochromator is the third component and its primary function is to select a particular wavelength of the element being determined and to isolate the absorption line from the background light. There is also a detector which is a photomultiplier tube capable of transforming radiation into an electrical current that can be easily measured. This detector measures the amount of light absorbed by the analyte and this amount of light is proportional to the concentration of that analyte in the sample. Lastly, signal processer computer system is used as a read out device to read the data from the detector and it is equipped with a WinLab32 software which has a Zeeman-effect background correction for correcting analyte signals for background attenuation.

2.5.2 The GFAAS used for the current research

The high performance AAnalyst 800 (figure 14) with a dedicated Transversely Heated Graphite Atomizer (THGA) manufactured by Perkin Elmer was used for the determination of the cadmium on the digested sewage sludge samples.



1 – Extractor fan, 2 – Graphite Furnace compartment , 3 – Auto sampler, 4 – Port (for control cable), 5 – Rinse bottle, 6 – Waste bottle, 7 – Lamps, 8 – Cooling system, 9 – Computer

Figure 14: The THGA Graphite Furnace instrument used for this current research

2.5.3 Operating conditions and specifications of the THGA graphite furnace

GFAAS characteristics

Manufacturer	Perkin Elmer
Model	THGA AAnalyst 600

Conditions sets

Wavelength (nm)	228.8
Replicates	3
Read time (s)	5
Low slit (nm)	0.7
Tube/Site	Pyro/Platform
Pre-treatment Temp (^{o}C)	700
Atomization Temp (^{o}C)	1400
Rollover (A)	0.8
Characteristic Mass	1.3 pg/0.004 A-s
Sensitivity Check	2.0 µg. L^{-1} for 0.13 A-s
Diluent	0.2% conc. HNO_3
Matrix Modifier	50 µg of $NH_4H_2PO_4$ & 3.0 µg of $Mg(NO_3)_2$

The matrix modifier solution used was prepared by weighing 1.0 g of the ammonium dihydrogen phosphate and 0.06 magnesium nitrate salts into a 100 ml volumetric flask and then made up to the mark with ultra-pure water to give a final concentration of 0.09 M and 0.004 M for the ammonium dihydrogen phosphate & magnesium nitrate salts respectively. The autosampler was set up to take 20 µl of the calibration standard solutions and samples as well as 5 µl of the matrix modifier (i.e. 50 µg of $NH_4H_2PO_4$ and 3.0 µg of $Mg(NO_3)_2$).

Table 9 below shows the recommended settings and/or conditions for each of the above steps (Perkin Elmer, 2004). The importance of optimizing each of these steps will be demonstrated as some changes were made on the temperatures in order to achieve the optimum conditions suitable for the detection and determination of the cadmium in the sewage sludge samples.

Table 9: The current furnace program used before optimization

Step	Temperature (^{o}C)	Ramp time (s)	Hold time (s)	Internal gas flow
1	100	1	30	250
2	130	15	30	250
3	700	15	20	250
4	1400	0	10	0
5	2500	1	5	250

2.5.4 Calibrating the graphite furnace

Before the graphite furnace was optimized, calibration standard solutions in the concentration range of 10 - 50 µg. L^{-1} were prepared and measured. The cadmium standard solutions were prepared through serial dilutions of a 1000 mg. L^{-1} stock solution purchased from Merk Chemicals. Each solution was diluted with concentrated HNO_3 in order to make a 0.2 % v/v final nitric acid concentration. This dilution with the HNO_3 was a recommendation from Perkin Elmer for the analysis of the cadmium in the graphite furnace. The calibration was performed before optimizing the furnace program in order to demonstrate the necessity of optimization. The calibration curve obtained showed a good linearity as shown in Figure 15 below. The blank solution measured was very low and this was not due to contamination but rather problems associated with the pyrolysis and atomization. Moreover, there appears to be problems with the readings as the line does not pass through the origin and this may be due to contamination or non-specific absorbance due to matrix effects. It was this point that a decision was made to try and optimize the procedure and reduce the high background reading.

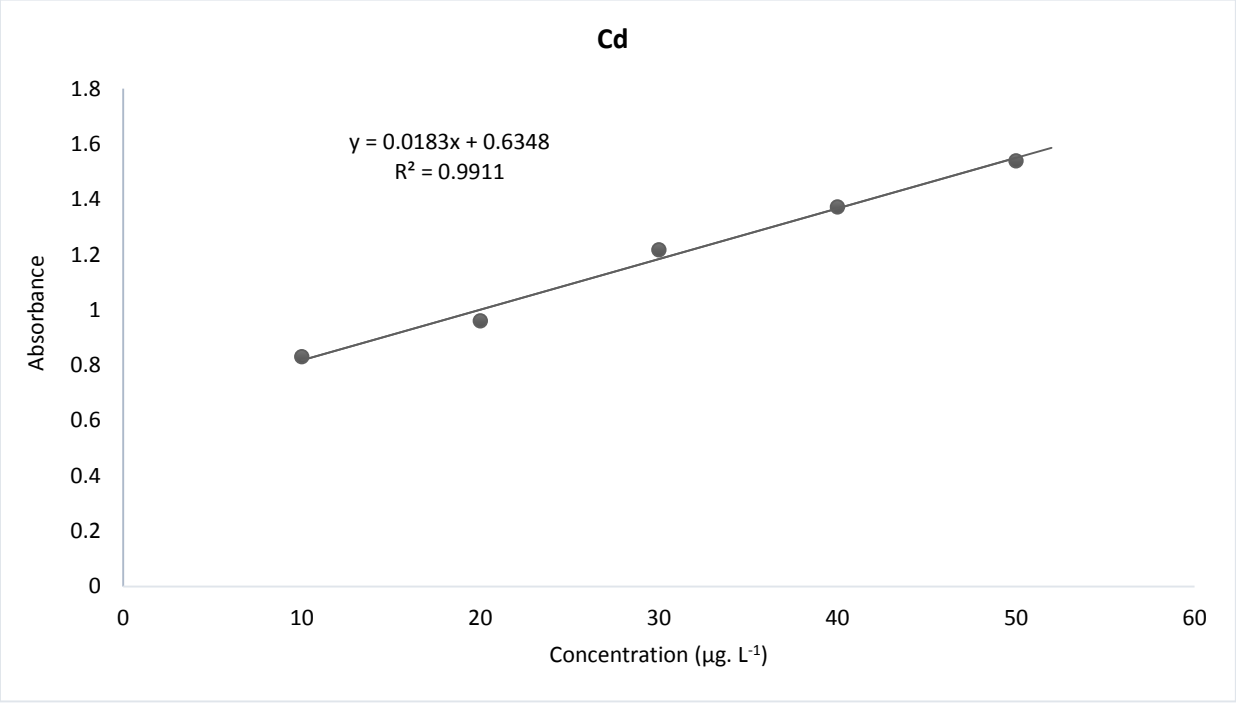


Figure 15: Calibration of the GFAAS before process optimization

2.5.5 Optimizing the graphite furnace

Using a cadmium standard solution

The temperatures of the pyrolysis and atomization steps were altered in order to find the most appropriate combination for both of these steps (see table 10). The test solution used for this trial and error experiment was a 50 µg. L^{-1} cadmium standard solution. The thermal pyrolysis step was used for removing most of the matrix before the atomization as well as to decrease the chances of chemical interferences and in turn reduce the amount of background signal. The recommended thermal pyrolysis temperature for the cadmium was 700 ^{o}C however other temperatures were investigated to broaden the scope in the development of an appropriate furnace program. These thermal pyrolysis temperatures were increased from 700 ^{o}C by 100 ^{o}C increments all the way to 1000 ^{o}C.

It was discovered that at a pyrolysis temperature of 1000 ^{o}C (regardless of the atomization) the absorbances were negative. The possible cause for negative absorbance readings was due to measuring the background when there was still some cadmium present in the optical path. At the end there was less cadmium present in the tube and thus a negative absorbance, see figures 18 & 19. The atomization step ensured that the cadmium was completely volatilized and the recommended temperature for this step was 1400 ^{o}C.

Table 10: Standard solution absorbance readings during the optimization of the furnace program

Solution	Temperature (^{o}C)		Mean Absorbance	S/N
	Pyrolysis	Atomization		
Standard (50µg/L)	800	1400	2.3422	1.59
	900	1400	1.0058	1.18
	1000	1400	-2.173	21.39
	1000	1500	-2.064	-87.87

The first temperature combination investigated was 800/1400°C for the thermal pyrolysis and atomization (represented by the white & blue traces on the spectrograms in figure 16 to 23) respectively. These temperatures gave the highest absorbance for the cadmium on the standard solution. The high absorbance of 2.3422 obtained for the standard solution caused a self-reversal on the absorption peak and as a result the peak shape changed, see the figure 16 below. The reason for the change of the peak shape was the concentration of the cadmium being too high.

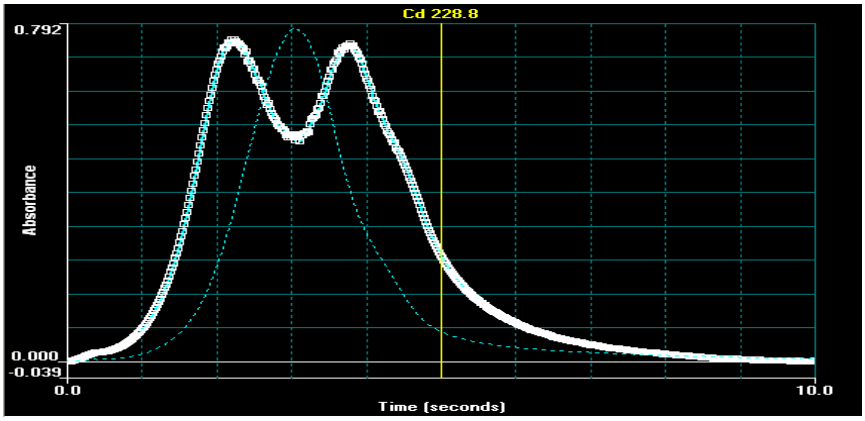


Figure 16: The absorption peak for the standard solution at 800/1400 °C

At the pyrolysis temperature of 900 °C there was no self-reversal observed as shown in figure 17 below despite having yet again a higher absorbance (i.e. > 1.00). The signal to noise ratio obtained at this temperature was above 1 and therefore based on this, the 900 °C thermal pyrolysis temperature was favourable.

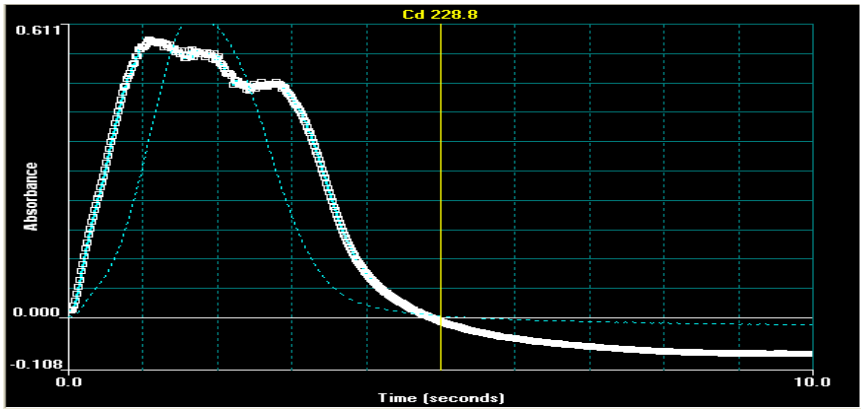


Figure 17: The absorption peak for the standard solution at 900/1400 °C

Obtaining negative absorbance readings at a thermal pyrolysis of 1000 °C for the standard solution (see figure 18) which was known to definitely have cadmium present and a mild self-reversal were the reasons this temperature was disregarded.

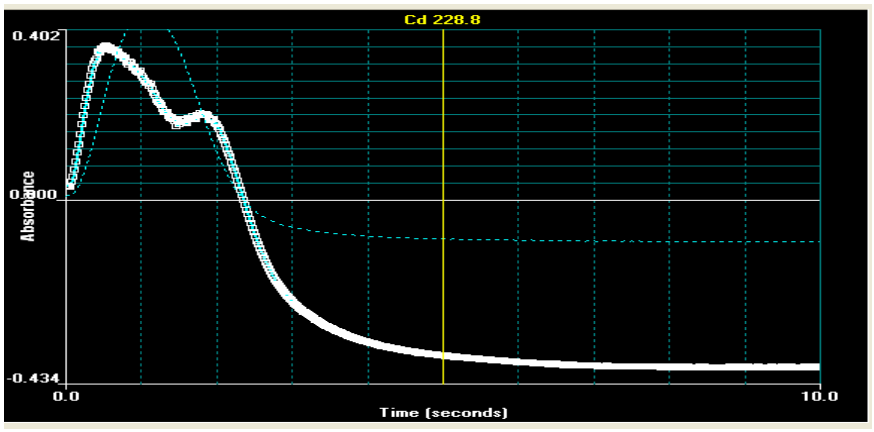


Figure 18: The absorption peak for the standard solution at 1000/1400 °C

When the atomization temperature was changed from 1400 °C to 1500 °C, the absorbance peaks extended further below the background noise, see figure 19 below. Furthermore, the signal to noise ratio obtained was negative and this meant that the background noise was significantly high enough to conclude that there was no cadmium detected at all.

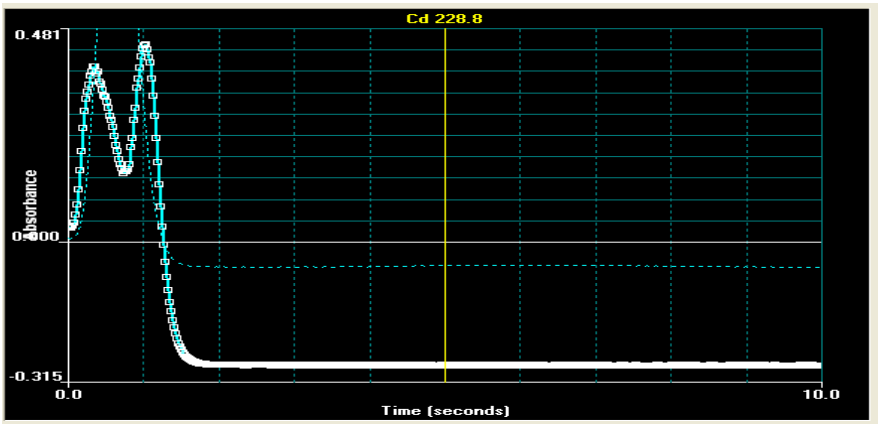


Figure 19: The absorption peak for the standard solution at 1000/1500 °C

A conclusion made based on these findings was that the combination of a thermal pyrolysis temperature of 900°C and an atomization temperature of 1400 °C gave the optimum results for the absorbance of the cadmium in the standard solution. Therefore these temperatures will be used for future measurements of the cadmium.

Using a digested & spiked sewage sludge sample

This experiment was performed in a similar manner as in section 2.5.5 with the main difference being the test solution which was 20 ml of the digested sewage sludge sample spiked with 5 ml of a 50 µg. L^{-1} cadmium standard solution to give an approximate final concentration of 10 µg. L^{-1} of cadmium. The cadmium was added to the sample for behavioral assessment within the sample matrix. The measured absorbances are given in table 11 below together with the signal to noise ratios obtained.

Table 11: Spiked sample absorbance readings obtained during the optimization of the furnace program

Solution	Temperature (°C)		Mean Absorbance	S/N
	Pyrolysis	Atomization		
Spiked sample	800	1400	0.191	2.09
	900	1400	0.059	1.02
	1000	1400	0.045	0.80
	1000	1500	-0.087	-2.47

Although the absorbance reading was not too high for the 800/1400 °C combination, the peak shape of the spiked sample however looks distorted and shows a mild self-reversal. Therefore the pyrolysis temperature of 800 °C was not favourable to use for further determinations of the cadmium on the graphite furnace.

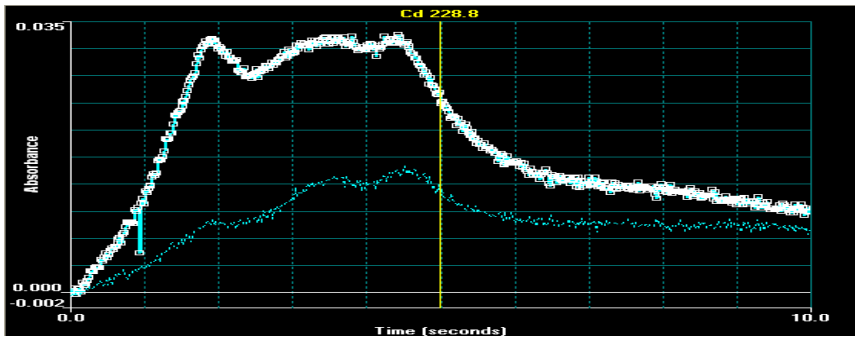


Figure 20: The absorption peak for the spiked sample at 800/1400 °C

At a 900/1400 °C combination the findings were very much similar to those of the standard solution and although the peak shape was not so perfect, there was an absorbance peak observed due to the cadmium. Figure 21 below shows the cadmium peak with no self-reversal and the absorbance was not negative meaning that the background was measured with no cadmium present.

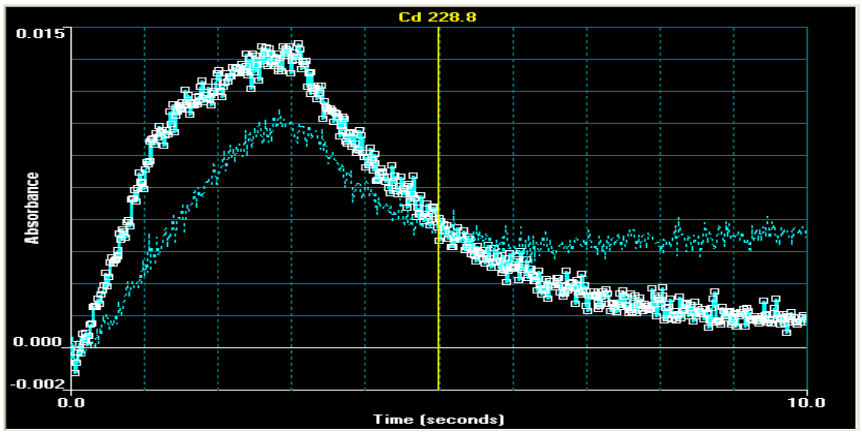


Figure 21: The absorption peak for the spiked sample at 900/1400 °C

The 1000/1400 °C combination in figure 22 was another possible candidate but the only concern was the low signal to noise ratio that was obtained. These findings led into disregarding these temperatures.

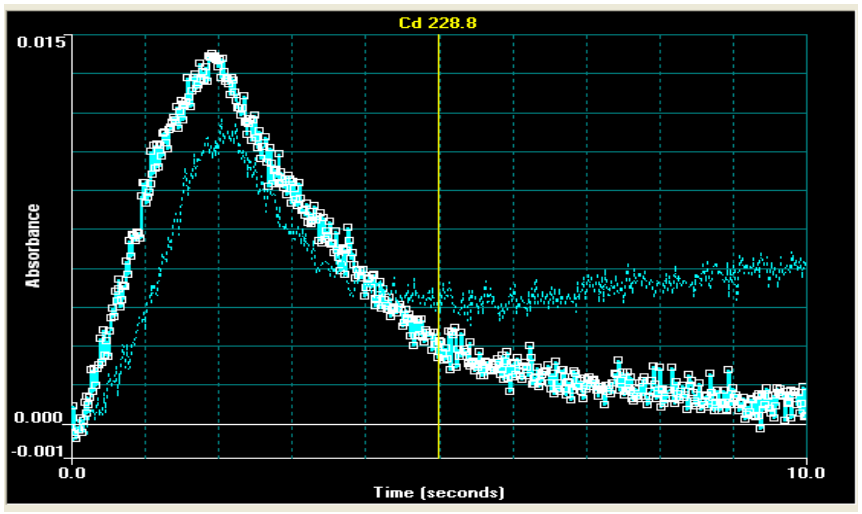


Figure 22: The absorption peak for the spiked sample at 1000/1400 °C

At the 1000/1500 °C combination in figure 23, the air ashing peak was higher than the signal for the absorbance of the cadmium. The possible reason for the negative absorbance was that at 1500 °C the spiked sewage sludge sample was volatilized and completely evaporated off therefore leaving no cadmium behind to absorb the incident light.

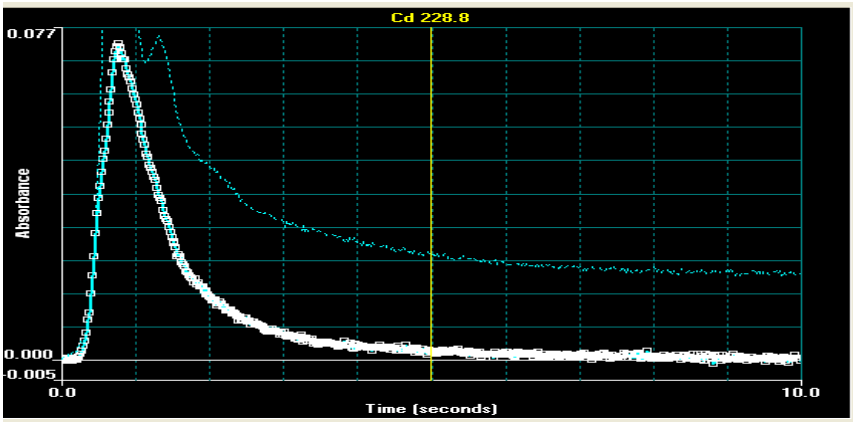


Figure 23: The absorption peak for the spiked sample at 1000/1500 ^{o}C

After considering all the findings made, a conclusion similar to that made for the standard solution was also made herein as the thermal pyrolysis temperature of 900^{o}C and an atomization temperature of 1400 ^{o}C gave the optimum results and were used going forward.

2.5.6 Calibrating the graphite furnace after optimization

The furnace program was set to 900/1400 °C thermal pyrolysis & atomization and the calibration was performed once again with the similar set of standard solutions. An improvement in the linearity of the calibration curve and better sensitivity of the method was obtained as shown in figure 24 below.

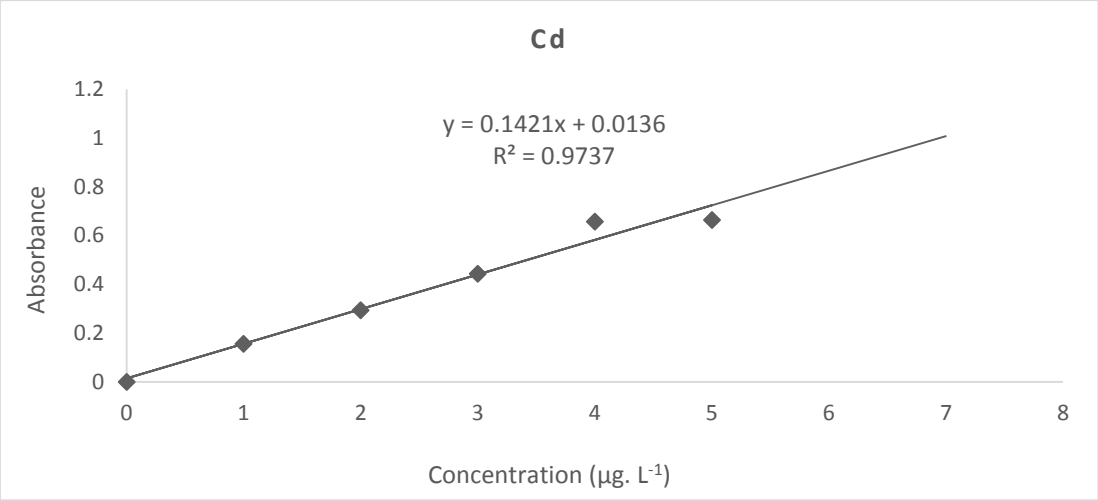


Figure 24: Calibration of the GFAAS after process optimization

2.5.7 Determination of cadmium using the newly developed furnace program

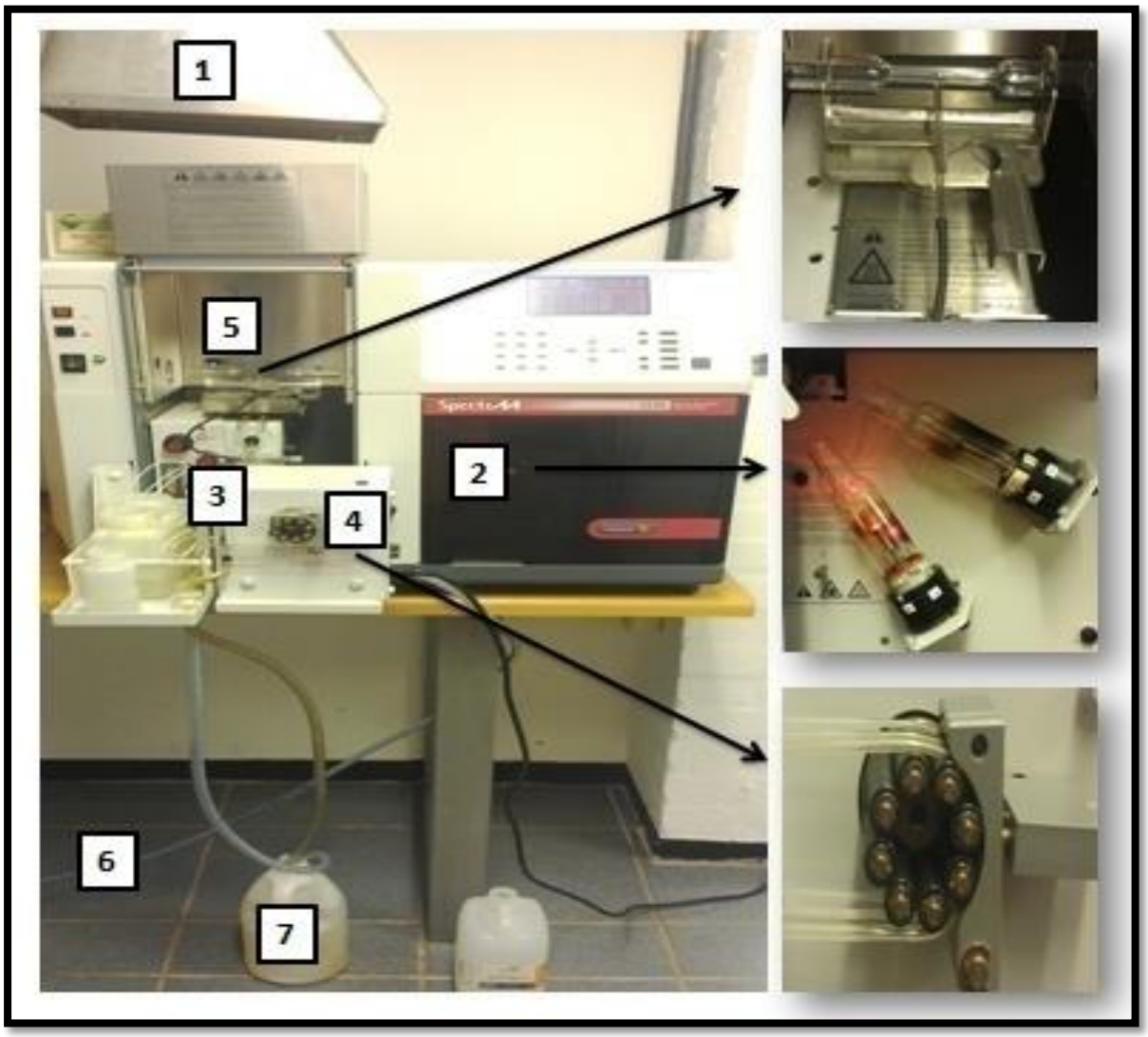
When the furnace program was developed, the calibration was then performed. Using the information from the calibration curve in figure 24 above, the concentrations of the digested sewage sludge samples were determined. These samples were prepared according the random sampling technique discussed in section 2.2.1. The concentration of cadmium in the samples was then calculated and reported in relation to the masses given in table 12 below, i.e. mg. kg^{-1}.

Table 12: The masses of the digested sewage sludge samples

Sample	Mass (g)
1	3.0082
2	3.0064
3	3.0050
4	3.0003

2.6 The VGA-77 with AA module used for this research

The Perkin Elmer VGA-77 for the vapour generation of mercury with installed AA module for the analysis of mercury concentration in the sewage sludge is shown in figure 25 below.



1 – Extractor fan, 2 – Mercury Lamp, 3 – VGA-77 for the Hg vapour generation, 4 – Pump rollers with pressure bar, 5 – Mark 7 burner with standard absorption cell, 6 – Hose connected to gas supply, 7 – Waste bottle.

Figure 25: The VGA-77 for cold vapour generation of mercury with installed AA module

2.6.1 The operating conditions and specifications of VGA-77 with AA module for mercury determination

VGA-77 characteristics	
Manufacturer	Varian
Model	VGA-77
Conditions sets	
Inert gas	Nitrogen
Pressure	350 kPa (Flow rate: 100 mL/min)
Sample uptake rate	6 – 8 mL/min
Reagents uptake rate	0.8 – 1.2 mL/min
Reductant	$SnCl_2$ (25% w/v) in HCl (20% v/v)
AA characteristics	
Manufacturer	Varian
Model	SpectrAA 50 BB
Conditions sets	
Lamp current	4 mA
Fuel	Acetylene
Support	Air
Wavelength (nm)	253.7
Slit width (nm)	0.5
Optimum working range (µg/L)	2 – 20
Measurement parameters	
Pre-read delay time (min)	1
Read time (s)	3
Replicates	3

2.6.2 Calibrating the VGA-77 with mercury standard solutions in 2% v/v HNO3

A total of five mercury standard solutions with a concentration range of 0 – 25 µg. L^{-1} to cover the potential concentration in the samples were prepared in 2% v/v HNO_3 and used to calibrate the VGA-77. The standard solutions were prepared through serial dilutions of a 1000 ppm stock Hg solution purchased from Merck Chemicals. The absorbance readings measured are plotted in figure 26 below.

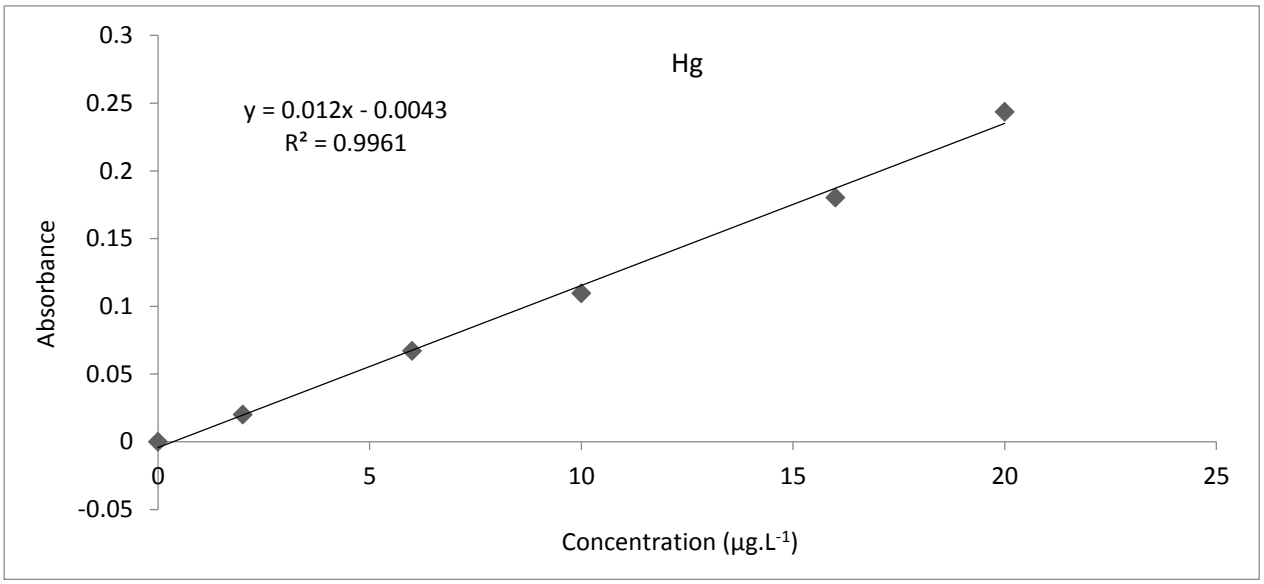


Figure 26: The calibration curve for the mercury standard solutions prepared in 2% v/v HNO_3

2.6.3 Calibrating the VGA-77 with standard solutions matrix matched to the samples

The ISO 11466 method specified that 7 ml of the HNO_3 must be used for the digestion and that the digestate must be made up to the mark of a 100 ml volumetric flask with a 0.5 mol. L^{-1} HNO_3 solution. The standard solutions prepared herein had an approximately similar concentration of the nitric acid as the digested sewage sludge samples. These were prepared by making a working standard of 1 ppm from the 1000 ppm stock solution. This working standard was diluted down to the concentrations in the range of 0 – 20 µg. L^{-1} by the addition of 7 ml of the concentrated HNO_3 and making up to the mark with a 0.5 mol. L^{-1} HNO_3 solution. The absorbance readings measured for the calibration solutions are shown below in figure 27.

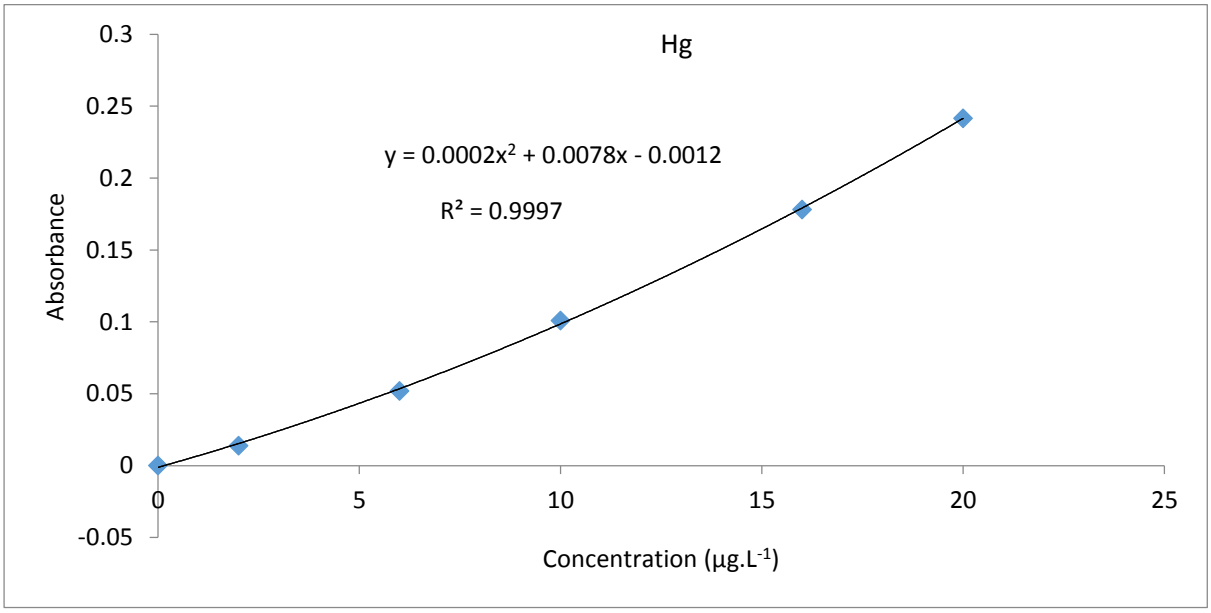


Figure 27: The calibration curve of matrix matched mercury standard solutions

2.6.4 Spiking the samples for method validation of the VGA-77 AA system

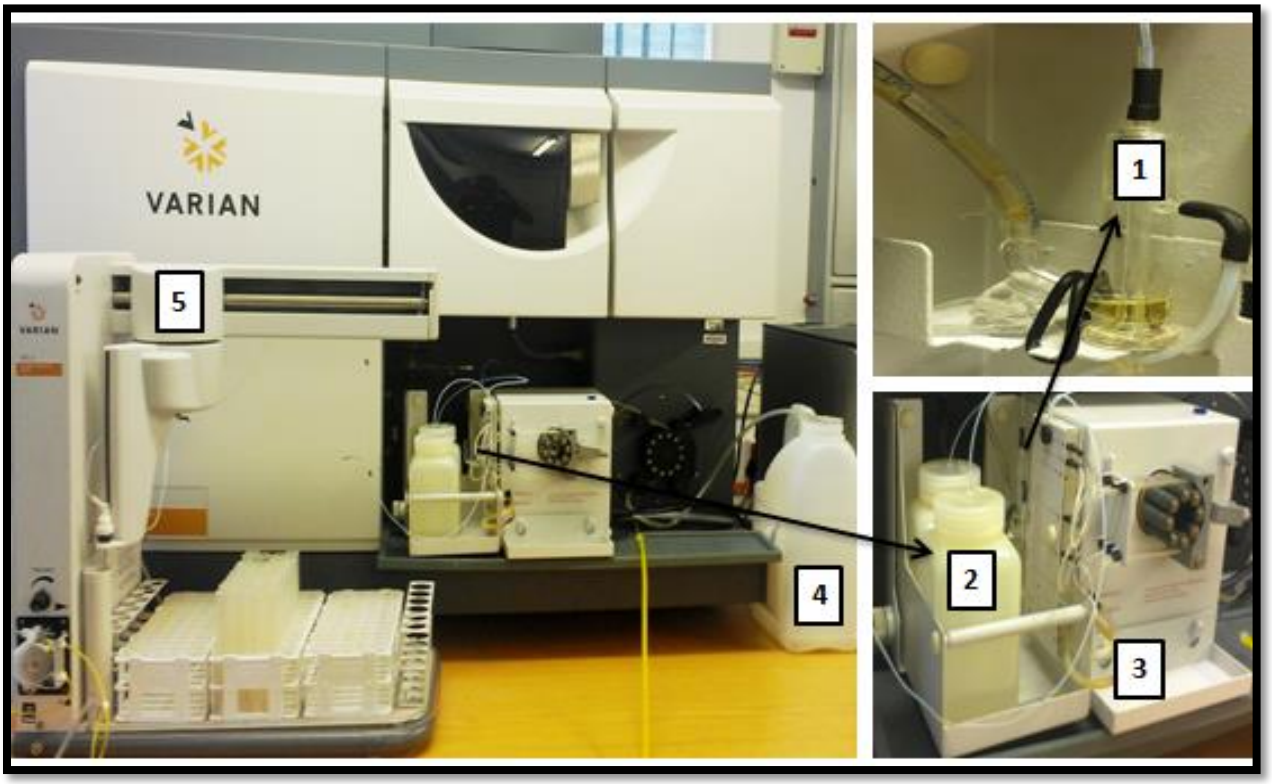
The spiking of the samples was performed in order to validate the VGA-77 AA system method used for the analysis of mercury content. The samples (25 ml of each) were spiked with 0.025 µg of mercury by adding 250 µL of a 100 $\mu g.L^{-1}$ standard solution to give a final volume of 25.25 ml of a spiked sample. The absorbances of the spiked samples were then measured on the VGA-77 AA system and the results are shown later in the results and discussion chapter.

2.6.5 VGA-77 ICP-OES system: An alternative to the VGA-77 AA system

It has been mentioned previously that chemical vapour generation can also be used together with optical emission spectrometry. This technique was also used for this research as a VGA-77 ICP-OES system was assembled for the analysis of mercury in the sewage sludge samples. The purpose of using two techniques for the same task was to establish the most sensitive and robust method which would be able to measure the concentration of mercury with a higher precision and certainty.

2.7 The VGA 77 - ICP system used for the current research

Perkin Elmer VGA-77 with installed ICP-OES module used for the analysis or determination of mercury in the sewage sludge is shown in figure 28. The operating conditions and specifications of the VGA-77 with ICP-OES module are given in section 2.7.1.



1 – Plasma gas/liquid separator, 2 – Reductant container, 3 – Argon inlet, 4 – Waste bottle, 5 – Autosampler

Figure 28: The VGA – 77 with installed ICP-OES module

2.7.1 The operating conditions and specifications of the VGA-77 with ICP-OES module

VGA-77 characteristics

Manufacturer	Varian
Model	VGA-77

Conditions sets

Inert gas	Argon
Pressure	350 kPa (Flow rate: 100 mL/min)
Sample uptake rate	6 – 8 mL/min
Reagents uptake rate	0.8 – 1.2 mL/min
Reductant	$SnCl_2$ (25% w/v) in HCl (20% v/v)

ICP characteristics

Manufacturer and Model	VARIAN 720-ES ICP-OES
Frequency	40 MHz
Power	1.00 kW

Conditions sets

Plasma argon flow rate (L/min)	15.0
Auxiliary argon flow rate (L/min)	15.0
Nebulizer flow (L/min)	0.75
Instrument stabilization time (s)	15
Replication time (s)	10
Pump rate (rpm)	35
Rinse time (s)	10
Sample uptake delay time (s)	35
Replicates	3

2.7.2 Calibrating the VGA-77 ICP-OES system mercury standard solutions

The mercury standard solutions used for calibrating the VGA-77 ICP-OES system were prepared in the similar manner as those of the VGA-77 AA system. The emission intensity readings measured for the standard solutions are plotted in figure 29 against the concentrations of the standard solutions. There is a good linear correlation and small reading for the blank. The value recorded for the blank represents a concentration of 0.8 µg. L^{-1} Hg in the blank and this could be improved by using higher purity acids.

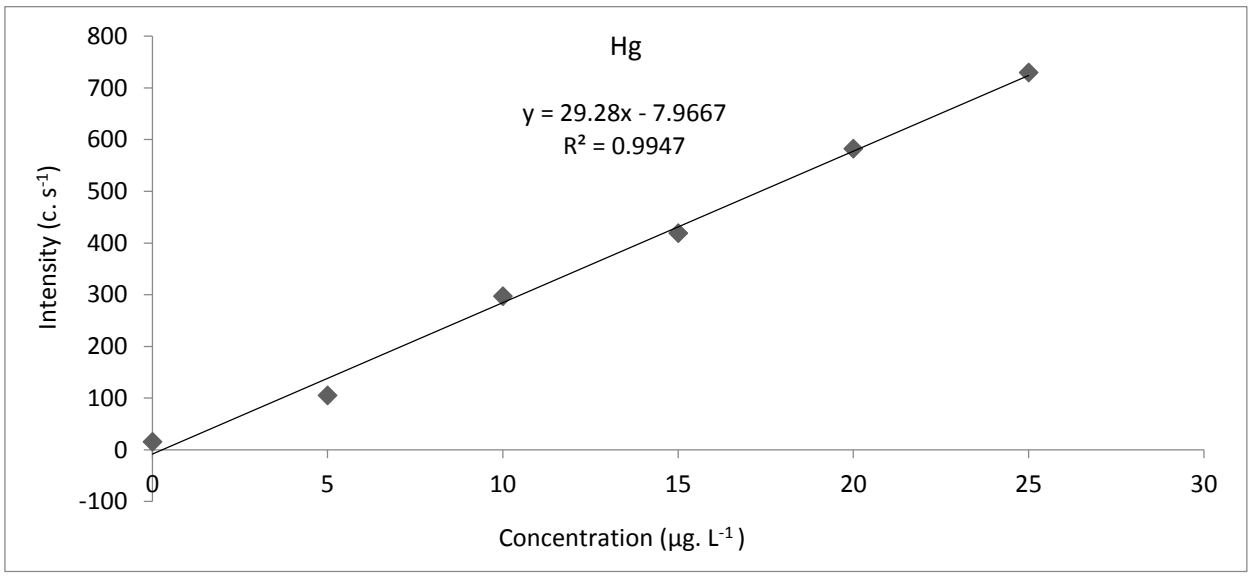


Figure 29: The calibration curve for the mercury standard solutions

2.7.3 Spiking the samples for method validation of the VGA-77 ICP-OES system

The spiking of the samples was similar to that of the VGA-77 AA system as the same mercury mass of 0.025 µg was added to the samples. This was done by adding 5 ml of the new working standard with a concentration of 5.00 µg. L^{-1} to give a final volume of 25 ml of a spiked sample. The absorbances of the spiked samples were then measured on the VGA-77 ICP-OES system and the results for this method validation experiment are in section 3.11.5.

CHAPTER 3

3. Results and Discussion

This chapter discusses the results obtained in the research work performed. The results of the experiments performed are discussed separately and following the order in which the experiments were performed as shown in Chapter two for Methodology. The cause and effect diagrams described in chapter one are a very convenient way of listing the sources of uncertainty, showing how they relate to each other and indicating their influence on the overall uncertainty of the result. The cause and effect diagram in figure 30 will be the tool used herein to map out the various contributions to the overall uncertainty of the concentrations of the analytes measured through different detection systems.

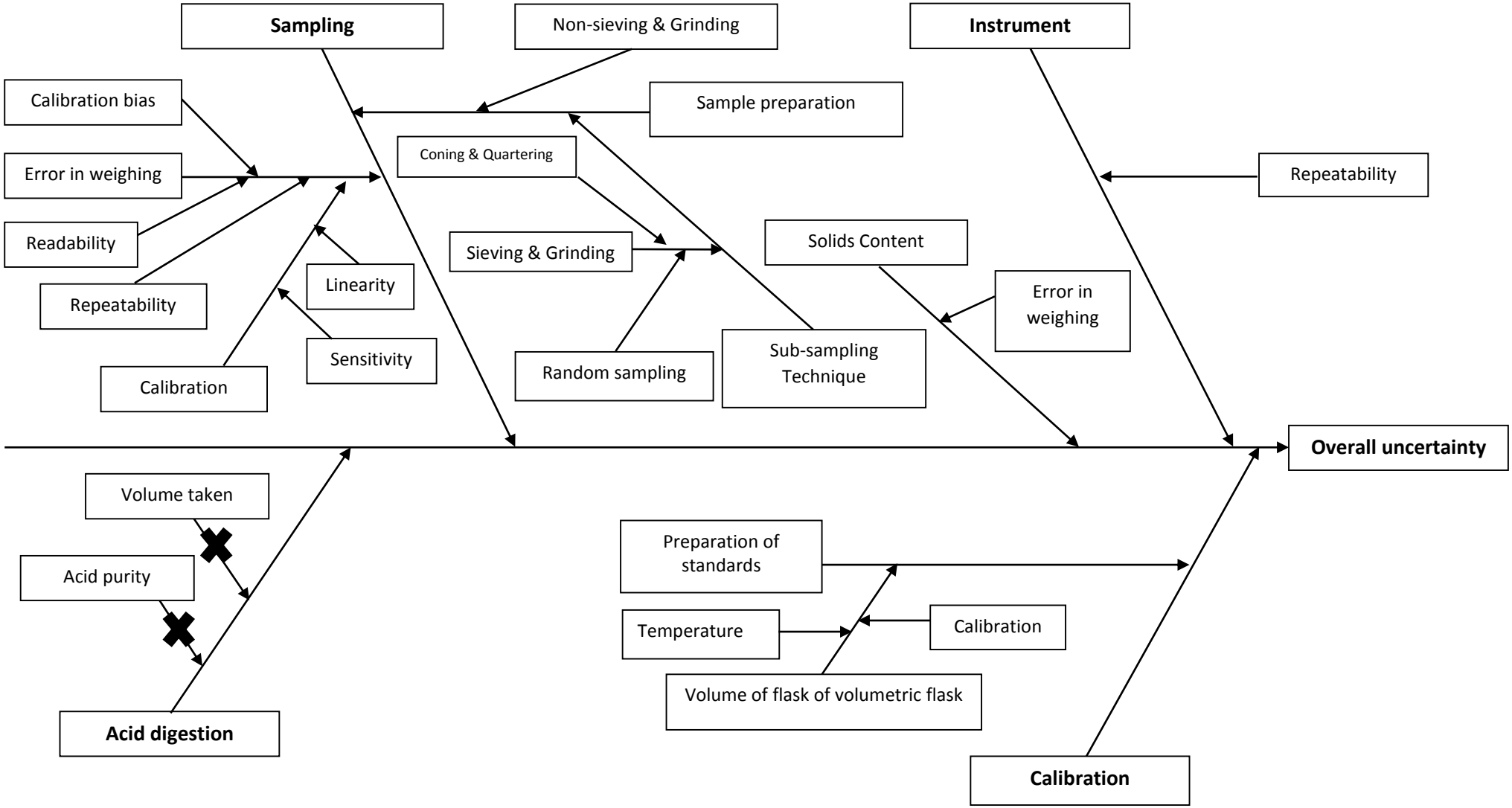


Figure 30: The cause and effect (Ishikawa) diagram for the determination of metal concentrations

3.1 Determination of Solids content in the sewage sludge

Prior to digestion, the solids content of the sewage sludge was determined following the procedure outlined in section 2.1. This procedure required the mass of the samples to be measured before and after oven drying. The moisture and solids content of the four subsamples were calculated as shown in Appendix A and the results are shown in the table 13.

Table 13: Solids content of the sewage sludge from oven drying at 100 ^{o}C

Mass before oven drying (g)	Mass after oven drying (g)	Mass loss on drying (g)	% Moisture content (w/w)	% Solids content (w/w)
3.0031	2.9611	0.0420	1.40	98.60
3.0089	2.9517	0.0572	1.90	98.10
3.0027	2.9697	0.0330	1.10	98.90
3.0093	2.9461	0.0632	2.10	97.90

The variation of the solids content in all the samples was calculated as the relative standard deviation and found to be 0.47%. This low relative standard deviation meant that the variability in the sample itself was very low. The measurement of mass in the determination of the solids content was subjected to a calibration uncertainty. This calibration of the mass balance entails creating a calibration curve of the balance's signal as a function of the mass. All calibration curves are subject to a systematic uncertainty in the y-intercept (bias) as well as the uncertainty in the slope (linearity) (Eurachem, 2012). Therefore based on this discussion, the error in weighing had to be estimated by following the four main steps involved in the process of measurement uncertainty estimation.

The majority of the experimental work performed required a mass of 3.0 g of the sewage sludge to be weighed. This mass was measured by tared weighing. In general, this form of weighing has three uncertainty sources namely the repeatability, the readability of the balance scale and the contribution due to the uncertainty in the calibration function of the scale (Eurachem, 2012).

This calibration function has two potential uncertainty sources which are the sensitivity and linearity of the digital mass balance, see figure 30. The contribution from the "Sensitivity" will be neglected because the mass by difference was done on the same balance. Moreover, this weighing by difference meant that the contribution from the "Calibration Bias" must be eliminated as the bias contributed equally to both gross mass (i.e. the mass of the samples before subtracting the tare value) and tare mass (i.e. the mass balance tare value) (Eurachem, 2012).

The calibration certificate of the balance was examined and the recommendation on uncertainty estimation was 0.10 mg. This recommendation on uncertainty will be used for the estimation of the uncertainty in weighing or sample mass of the sewage sludge. This estimate takes into account the three contributions mentioned earlier (i.e. repeatability, readability and calibration function). It is worth noting that there's usually several contributions of uncertainty to weighing and some of these include for example "Daily Drift" which itself has several causes like temperature variations. There is also "Density effects" resulting from calibration weight/sample density mismatch which causes a difference in the effect of atmospheric buoyancy (Eurachem, 2012). These contributions to uncertainty are too small consider and will not be quantified and accounted for herein.

In general, detailed calculations of the uncertainties in mass can be very complicated and it is important to refer to the manufacturer's literature (if available) where mass uncertainties are dominant. Therefore, for the purpose of this research the error in weighing will be estimated and it will be shown that the variability of the solids content, although very small, was far higher than the variability of the weighing.

The error in weighing for the determination of solids content was calculated as the relative standard uncertainty $\frac{u(x)}{x}$ based on the manufacture's standard uncertainty of 0.10 mg denoted as $u(x)$ in table 14. The masses of the samples were weighed before and after oven drying at 100 ^{o}C, therefore the uncertainty of 0.10 mg had to be propagated in order to contribute equally to the masses weighed. To combine these uncertainties in a propagation, a simplified expression shown in equation 1 was used. This expression for uncertainty combination is for models involving only a sum or difference of quantities, e.g. y= (p + q +...) (Eurachem, 2012), see Appendix C for other uncertainty combination rules.

$$u_c\left(y(p,q..)\right) = \sqrt{u(p)^2 + u(q)^2 + \ldots}$$

Equation 1: The model for uncertainty combination involving only a sum of quantities

Therefore, based on equation 1 the uncertainty in weighing was expressed as follows:

$$\text{Uncertainty in Weighing} = \sqrt{((0.10)^2 + (0.10)^2)}$$

$$= \underline{0.14 \text{ mg}}$$

Table 14: Estimating the uncertainty in the weighing for solids content determination

Sample	Solids (g)	Standard uncertainty $u(x)$ (g)	Relative standard uncertainty $\frac{u(x)}{x}$
1	2.9611	0.00014	0.00004728
2	2.9517	0.00014	0.00004743
3	2.9697	0.00014	0.00004714
4	2.9461	0.00014	0.00004752

The average relative standard uncertainty for weighing all the samples was calculated to be 0.0047%. These findings showed that weighing had such a small error compared to the error in the variability of the solids content of the samples. The contributions from the variability in the solids content and the error in weighing will be used to calculate the overall expanded uncertainty.

3.2 The sources of uncertainty in the acid digestion

The "Purity of the Acids" was first noted as a possible contribution to the uncertainty in the acid digestion. Two of the acids used in the aqua regia were; 32% Hydrochloric acid & 65% Nitric acid which their certificates of analyses showed the following levels of impurities:

Table 15: The impurities in the acids used for aqua regia digestion (Merck Millipore, 2016)

Impurity	32% HCl	65% HNO_3
As	≤ 0.01 ppm	≤ 0.010 ppm
Cd	≤ 0.01 ppm	≤ 0.0010 ppm
Cr	≤ 0.02 ppm	≤ 0.020 ppm
Pb	≤ 0.01 ppm	≤ 0.010 ppm

There were several other impurities on the certificate of analysis and the ones shown in the table 15 above were of interest to this research as these were elements measured in the sewage sludge. The acids were analytical reagent grade and the levels of the impurities present in them were very low compared to the concentrations of these metals in the sewage sludge sample. The impurities of the acids were not expected to have an effect on the precision of the analyte concentrations unless different volumes of the acids were used. Using 21 ml of the 32% HCl with an arsenic impurity of 0.01 mg. L^{-1} results into adding 0.21 µg of the arsenic.

Moreover, a 3.0 g sample with an arsenic concentration of 14.02 mg. kg^{1} will have 42 µg of arsenic and an additional 0.21 µg if the acid has maximum impurity. A mass percentage expression, this equates to 0.48% which is actually a bit high to disregard totally. Therefore contributions from acid impurities will be to accuracy which will leads to bias. It should be mentioned that if the impurities have an effect on accuracy then confirmation by measuring a certified reference material (CRM) must be performed but unfortunately this CRM was not available at the time this work conducted. The impurities of the acids were given without uncertainties and a confidence level at which they were measured meaning that these amounts represent a maximum. It is for this reason that the effect of impurities on the precision will be neglected and therefore crossed out from the cause and effect diagram.

The second possible contribution was the “Volume of the burette” used in the preparation of the calibration standards. The volume of the burette was subjected to three sources of uncertainty which were the uncertainty in the ‘’certified internal volume of the burette’’, the ‘’uncertainty in the variation in filling the burette’’ and also the ‘’uncertainty in the burette and solution temperatures differing from the temperature at which the volume of the burette was calibrated at’’ (Eurachem, 2012).

The variations in the volume of the acid delivered will affect the precision but should be very small when calculated as the manufacturer quoted the burette to a volume of (50.00 ± 0.01) ml at 95% confidence level. Therefore it was for this reason that the contribution from the volume of the burette was disregarded as it would matter only if the impurities from the acids were being included in the uncertainty budget.

3.3 Quantifying the uncertainty in the calibration of the ICP-OES

The calibration of the ICP-OES was one of the potential sources of uncertainty in the overall concentration of the metals in the sewage sludge. The volume of the volumetric flask used in the preparation of the standard solutions was a potential source of uncertainty in the calibration of the instrument. A high variability in the final volumes of the standard solutions can be expected to affect the accuracy and the variability in delivering volumes would in turn affect the precision. These variation effects can be shown in the slope, intercept and correlation coefficient of the best fit line of the calibration graph.

The following section discusses how the volume of the volumetric flask can influence the variation of the measurement of the calibration standards in the instrument on a worst case scenario. As it will be shown, the error associated with the volume of the volumetric is actually very small when expressed as %RSD but it is important & its contribution to the overall uncertainty is worth accounting for.

3.3.1 The volume of the volumetric flask (V)

The calibration standard solutions were prepared in 100 ml volumetric flasks and the variability in temperature has an effect in the volume contained in the flask which also has an effect of changing the calibration of the flask (Eurachem, 2012).

(i) Calibration of the volumetric flask

For the calibration (Cal), the flasks were quoted with a volume of 100 ml ± 0.1 ml measured at 20 ^{o}C. This uncertainty was given without a confidence level and in this case, the standard uncertainty was calculated assuming a triangular distribution. The triangular distribution forms part of the three most important distribution functions for calculating standard uncertainty (Eurachem, 2012). The other two distribution functions are the rectangular & normal distributions, refer to Appendix C for a thorough description of these distribution functions.

The triangular distribution can be used when the values close to x are more likely than near the bounds and it can also be used when an estimate is made in the form of a maximum range ($\pm a$) described by a systematic distribution, see equation 2 (Eurachem, 2012).

$$u(x) = \frac{a}{\sqrt{6}}$$

Equation 2: The estimation of uncertainty using a triangular distribution

For the calibration of the volumetric flask, the triangular distribution was between the limits of ± 0.1 ml in which the most probable result was at the center of the distribution and decreasing linearly towards each limit (Eurachem, 2012). Therefore, the standard deviation for a triangular distribution was estimated as follows:

$$u(Cal) = \frac{0.1}{\sqrt{6}}$$

$$= \underline{0.041}$$

(ii) Temperature variation of the flask

The 100 ml volumetric flasks used were specified by the manufacturer to have been calibrated at a temperature of 20 ^{o}C and the room temperature of the laboratory varied between the limits of ± 5 ^{o}C. The uncertainty resulting from this was calculated through the estimation of the temperature range and the coefficient of the volume expansion. The volume expansion of the liquid was the only effect that needed consideration due to the fact the volume of expansion of the liquid was larger than that of the flask (EURACHEM, 2012). The volume variation of the flask was calculated using equation 3 below:

Volume variation = ± [Volume x Temperature limits x Coefficient of volume expansion for water]

Equation 3 : The estimation of volume variation (Eurachem, 2012)

The coefficient of volume expansion for water at 20 ^{o}C is 2.07 x 10^{-4} $^{o}C^{-1}$ (Elert, 2015) and therefore leading to a volume variation of:

Volume variation = ± [100 ml x 5 ^{o}C x 2.07 x 10^{-4} $^{o}C^{-1}$]

= ± 0.104 ml

The estimation of the uncertainty for the temperature variation of the flask (T) was calculated using a rectangular distribution because an estimate of the volume variation was made in the form of a maximum range $(\pm a)$ with no knowledge of the shape of the distribution, see equation 4 (Eurachem, 2012).

$$u(x) = \frac{a}{\sqrt{3}}$$

Equation 4: The estimation of uncertainty using a rectangular distribution

Therefore, the uncertainty in the temperature variation of the flask (T) then becomes:

$$u(T) = \frac{0.104}{\sqrt{3}}$$

$$= 0.060\ ^{o}C$$

The uncertainty in the "volume of the flask $u(V)$" was estimated through the combination of the uncertainties in the "calibration $u(Cal)$'' with the uncertainty in the "temperature variation of the flask $u(T)$" using an expression similar to equation 1 because this estimation involved the sum of the quantities, see equation 5.

$$u(V) = \sqrt{(u(Cal))^2 + (u(T))^2}$$

Equation 5: The estimation of the uncertainty of the volume of the flask

The uncertainty of the volume of the flask was then calculated as:

$$u(V) = \sqrt{((0.041)^2 + (0.060)^2)}$$

$$= 0.073\ ml$$

The relative standard uncertainty resulting from this expression is given in table 16.

Table 16: The relative standard uncertainty in the volumetric flask

Description	Value (x) ml	Standard uncertainty $u(x)$ ml	Relative standard uncertainty $\frac{u(x)}{x}$
Volume of the flask, *V*	100.0	0.073	0.00073

The relative standard uncertainty in the volume of the volumetric flask was used as the only contribution to the uncertainty in the calibration of the ICP-OES and was calculated to be 0.073%.

3.4 ICP-OES reproducibility and contribution to uncertainty budget

The reproducibility of the ICP-OES and its contribution to the uncertainty budget was determined through a repeatability experiment using a mixed standard solution. This standard solution was measured three times on the ICP-OES. The average concentrations and their respective standard deviations are given in table 17.

Table 17: The values and uncertainties in the Instrument's reproducibility

Description	Concentration, (x) (mg. L^{-1})	Standard uncertainty, $u(x)$ (mg. L^{-1})	Relative standard uncertainty	
			$\frac{u(x)}{x}$	%
Repeatability of Arsenic, R_{As}	0.4459	0.0039	0.0087	0.87
Repeatability of Cadmium, R_{Cd}	0.9867	0.0087	0.0088	0.88
Repeatability of Chromium, R_{Cr}	0.4735	0.0032	0.0068	0.68
Repeatability of Lead , R_{Pb}	0.9439	0.0033	0.0035	0.35

The repeatability experiment showed that ICP-OES reproducibility was expected to be the main contributor to the overall uncertainty based on the high relative standard uncertainties obtained for each of the elements.

3.5 Quantifying the uncertainty in the sub-sampling

The physical sample preparation comprises the step from the field sample to the laboratory sample. For this research, only laboratory samples were available and there was no information given on how these were sampled. Nevertheless, different sub-sampling techniques were implemented as indicated in the methodology.

3.5.1 The uncertainty in weighing for the sub-sampling techniques

It has been mentioned before that 3.0 g of the subsamples were weighed from each of the sub-sampling strategies performed. The approach used before for quantifying the error in weighing for solids content determination was also used herein and the resulting uncertainties are shown in table 18.

Table 18: Estimating the uncertainty in the weighing of random sampling

Sample	Value (x) (g)	Standard uncertainty $u(x)$ (g)	Relative standard uncertainty $\frac{u(x)}{x}$
1	3.0041	0.00014	4.6603E-05
2	3.0044	0.00014	4.6598E-05
3	3.0040	0.00014	4.6605E-05
4	3.0033	0.00014	4.6615E-05

The average relative standard uncertainty or %RSD in weighing for the random samples was calculated to be 0.00466% and it is the same error obtained for the weighing for solids content. This finding was expected as the same procedure and analytical balance was used.

3.6 Quantifying the uncertainty on Sieved & Ground samples

Sieving and grinding was a mechanical treatment expected to have a small contribution to the overall uncertainty budget due to an increase in the number of particles and therefore reducing error. The process can however increase error if sample is lost by dust, segregation of different particle fractions or selectively grinding softer parts of the sample.

(i) Coning and Quartering

The coning and quartering was a random sub-sampling technique in which the lot was divided into N sub-lots of equal sizes and within each sub-lot, a sampling point was randomly assigned (EURACHEM, 2007). The standard deviations of the concentrations (see table 19 below) of the analytes obtained from coned & quartered sewage sludge samples were used in the estimation of the relative standard uncertainties.

Table 19: The concentrations of the analytes obtained from coned & quartered subsamples

Sample	As (mg. kg^{-1})	Cd (mg. kg^{-1})	Cr (mg. kg^{-1})	Pb (mg. kg^{-1})
1	14.40	19.91	15.39	30.68
2	13.59	20.22	15.98	31.12
3	14.94	19.67	16.06	31.31
4	14.64	19.91	15.06	30.40
Mean	14.39	19.93	15.62	30.88
SD	0.58	0.23	0.48	0.41
%RSD	4.02	1.13	3.07	1.34

These results showed that for all the analytes, the variability in the coned and quartered samples was different & higher than the instrument's reproducibility, see the F-test in table 20.

Table 20: Comparing precision of the ICP-OES reproducibility against coning & quartering using F-test

Analyte	ICP-OES reproducibility %RSD	Sieved & Ground sample	F-test		
		Coning & Quartering %RSD	F_{stat}	F_{crit}	P_{value}
As	0.87	4.02	21.35	9.28	0.016
Cd	0.88	1.13	1.65	9.28	0.34
Cr	0.68	3.07	20.38	9.28	0.017
Pb	0.35	1.34	14.66	9.28	0.027

The F-test calculations showed that the precision of the ICP-OES was significantly different for all the analytes with an exception of the cadmium when compared to the precision of the coned & quartered sieved & ground samples. The probability values calculated for the As (at 99.98% CI), Cr (at 99.98% CI) and Pb (at 99.97% CI) using 3 degrees of freedom for the numerator and denominator were all < 0.05 yet again meaning that the differences in the precision were significant. It is worth mentioning here that all statistical calculations would be based on 95% confidence level from here onwards. For cadmium, a different case was observed as the instrument's precision was shown not to be significantly better than the coning and quartering of the sieved & ground samples at 99% CI. This finding was expected due to a possible interference that was observed in the emission line of the cadmium. The same test was performed for sieved & ground random subsamples, see table 22.

(ii) Random Sampling

The random sub-sampling technique required no coning & quartering of the sieved & ground sample. The four subsamples were taken directly from the sieved & ground lot. As it was for the coning & quartering discussed above, the standard deviations of the analytes obtained from random samples were used to calculate the relative standard uncertainties, see table 21 below.

Table 21: The concentrations of the analytes obtained from the random sampling

Sample	As (mg. kg^{-1})	Cd (mg. kg^{-1})	Cr (mg. kg^{-1})	Pb (mg. kg^{-1})
1	14.28	19.94	15.94	30.52
2	14.32	20.22	15.64	30.76
3	14.54	19.66	15.85	30.71
4	14.64	19.92	15.21	30.61
Mean	14.45	19.94	15.66	30.65
SD	0.17	0.23	0.33	0.11
%RSD	1.20	1.15	2.08	0.35

Table 22: Comparing relative precision of the ICP-OES reproducibility against random sampling using F-test

Analyte	ICP-OES reproducibility %RSD	Sieved & Ground sample	F-test		
		Random sampling %RSD	F_{stat}	F_{crit}	P_{value}
As	0.87	1.2	1.90	9.28	0.30
Cd	0.88	1.15	1.71	9.28	0.34
Cr	0.68	2.08	9.36	9.28	0.05
Pb	0.35	0.35	1.00	9.28	0.50

The instrument's precision was not significantly different to the precision of the random samples as all the statistical F values were smaller than the critical F values for the arsenic, cadmium and lead at $\alpha = 0.05$. The statistical F value for chromium was slightly higher than the critical value and this discrepancy was observed only for this analyte. Therefore, since the precision of the instrument and for random samples was not significantly different, a conclusion made was that random sampling of the sieved and ground sample was ideal for ICP-OES analysis of the sewage sludge.

3.6.1 Comparing the precision between coned & quartered against random samples

The F-test in table 23 was used to compare the precision between the two subsampling techniques, cone & quartering and random sampling of the sieved & ground samples. The F-test was performed for each of the analytes in the four samples analysed.

Table 23: Comparing relative precision of the coning & quartering against random sampling using F-test

Analyte	Sieved & Ground sample		F-Test		
	%RSD Coning & Quartering	%RSD Random sampling	F	F_{crit}	P_{value}
As	4.02	1.2	11.22	9.28	0.04
Cd	1.13	1.15	1.04	9.28	0.49
Cr	3.07	2.08	2.18	9.28	0.27
Pb	1.34	0.35	14.66	9.28	0.03

The F-test comparison of the two subsampling techniques showed different variations. The differences in the precision of the techniques was significant for the arsenic and lead at 95% confidence level as the P values were less than $\alpha = 0.05$. The precisions of the cadmium and chromium were not significantly different at 95% CI. These findings suggested that for cadmium & chromium, any of the subsampling techniques would be appropriate to use. Furthermore, the mean concentration values of the two subsampling techniques were compared using the t-Test (two-sample assuming unequal variances) and the results showed that the difference was not significant at 95% confidence level, see Appendix B. It was discovered that although the precisions differed significantly (i.e. As and Pb) and insignificantly (Cd and Cr), the mean concentration values for all the analytes on the two subsampling techniques were shown not to differ significantly at 95% confidence interval.

3.7 Quantifying the uncertainty in non-sieved & ground samples

This sampling technique was straight forward and simple as the four subsamples were taken randomly from the plastic bag. The purpose of non-sieving and non-grinding was to assess the effect and significance of sample preparation from the sieved and ground random samples encountered in section 3.6. The standard deviations of the concentrations as well as the % relative standard deviations are reported in table 24 below.

Table 24: Analyte concentrations from the random sampling of non-sieved & non-ground samples

Sample	As ($mg.\ kg^{-1}$)	Cd ($mg.\ kg^{-1}$)	Cr ($mg.\ kg^{-1}$)	Pb ($mg.\ kg^{-1}$)
1	14.36	25.92	19.11	47.82
2	15.38	25.97	19.44	50.28
3	14.93	20.62	19.73	49.05
4	13.45	24.57	22.03	49.43
Mean	14.89	24.27	20.08	49.15
SD	0.51	2.52	1.33	1.02
%RSD	3.43	10.38	6.60	2.08

A general observation made for all the analytes was that the relative errors in the mean concentrations were higher for non-sieved & non-ground samples. These findings were expected because sieving & grinding was meant to reduce errors. As it was for the sieved & ground subsampling techniques already discussed, the precision of the ICP-OES repeatability was compared with the non-sieved and ground samples using the F-test, see table 25.

Table 25: Comparing the relative precision of the ICP-OES reproducibility against non-sieved & non-ground sub-sampling using F-test

Analyte	ICP-OES reproducibility %RSD	Non-Sieved & Ground sample	F-test		
		Random sampling %RSD	F_{stat}	F_{crit}	P_{value}
As	0.87	3.43	15.54	9.28	0.025
Cd	0.88	10.38	139.13	9.28	0.001
Cr	0.68	6.6	94.20	9.28	0.0018
Pb	0.35	2.08	35.32	9.28	0.0077

The precision for the non-sieved & non-ground subsamples was the worst compared to the other subsampling techniques. The F-test performed showed that the statistical F values for all the analytes were higher than the critical F values suggesting that the difference in the precision of the instrument was significantly different to the non-sieved & non-ground subsamples. To ascertain further, the precision for the cadmium worsened and this reiterated the findings made earlier about a possible interference.

3.7.1 Comparing the precision of coned & quartered samples against non-sieved & non-ground random samples

The non-sieved & non-ground subsamples were randomly sampled from the lot as explained before. Therefore to compare the precision and the effect of sieving & grinding, the random samples of the sieved & ground lot must be compared with the random samples of the non-sieved & non-ground lot using the F-test, see table 26. The means have already been shown not to be significant for coned & quartered as well random samples for the sieved & ground lot using the paired t-test, these can then be combined & their precision tested against non-sieved & non-ground subsamples.

Table 26: Comparing precision of the sieved & ground against non-sieved & non-ground samples

Analyte	%RSD Sieved & Ground	%RSD Non-Sieved & Non-Ground	F-Test		
			F	F_{crit}	P_{value}
As	1.2	3.43	8.17	9.28	0.06
Cd	1.15	10.38	81.47	9.28	2.30E-03
Cr	2.08	6.6	10.07	9.28	0.04
Pb	0.35	2.08	35.32	9.28	7.70E-03

The precisions were found to be significantly different for all of the analytes with an exception of the arsenic which had a probability value > 0.05. This finding was expected as it has been mentioned that sieving and grinding was meant to reduce errors and this was shown to be the case.

3.7.2 Comparison of overall precision using the F-test

Table 27 below groups together all the *P* values obtained through performing F-tests to compare the precision of the subsampling techniques. The probability values shaded in grey (see table 27) represent cases where the precision differed significantly (i.e. < 0.05). As it can be seen, the non-sieved sample was quite different in terms of precision compared to the sieved sample with the exception of arsenic which had close probability values for the two subsample preparations.

Table 27: Comparing precision of the sieved & ground against non-sieved & non-ground samples

Analyte	F-test (95% Confidence) *p* value	
	Coning & Quartering vs Random sampling Sieved & Ground	**Random sampling** Sieved & Ground vs Non-sieved & non-ground
As	0.04	0.06
Cd	0.49	2.30E-03
Cr	0.27	0.045
Pb	0.03	7.70E-03

3.8 Calculating the overall uncertainties

The subsampling errors already quantified (see table 28 below) encompass the analytical errors and will therefore be used directly as relative standard uncertainties in the calculation of the overall uncertainties of the measured concentrations. The graphical representation of the overall sampling errors expressed as %RSD is shown in Figure 31.

Table 28: Listing all the sub-sampling errors for the analytes

Element	%RSD Sub-sampling techniques		
	Sieved & Ground		Non-sieved & Non-ground
	Coned & Quartered	Random Sampling	
As	4.02	1.2	3.43
Cd	1.13	1.15	10.38
Cr	3.07	2.08	6.6
Pb	1.34	0.35	2.08

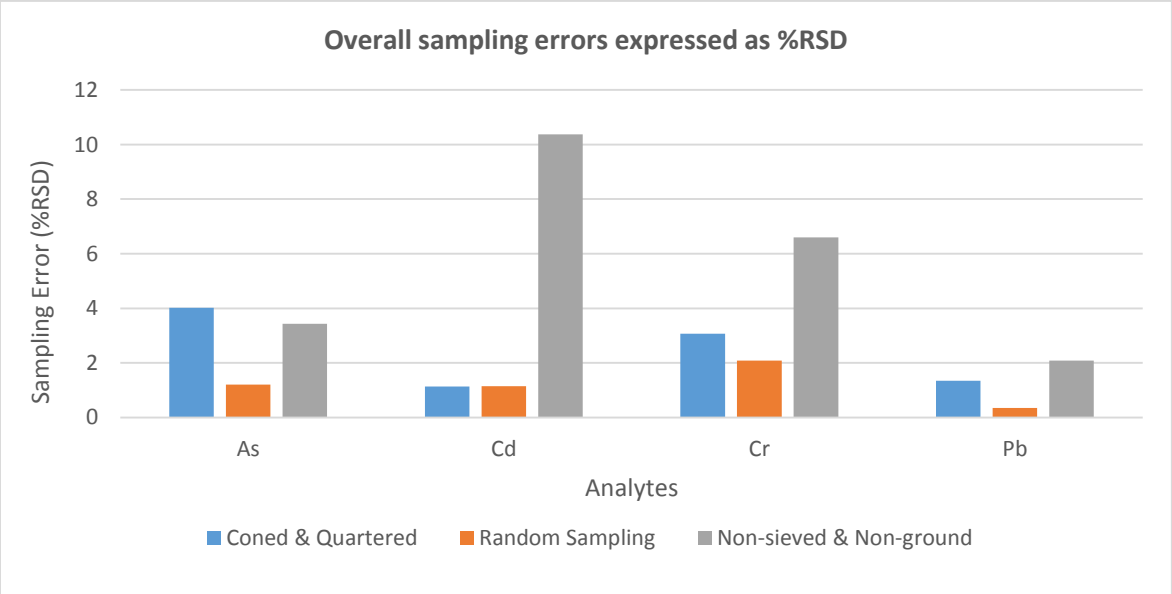


Figure 31: Column chart diagram showing the sampling errors obtained from the techniques

The overall combined uncertainty for all the analytes was calculated using equation 6:

$$\text{Standard uncertainty } u(x) = \text{Concentration } (x) \text{ x Relative standard uncertainty } \frac{u(x)}{x}$$

Equation 6: The calculation for overall combined uncertainty

For example, the overall combined uncertainty for the arsenic (Coned & Quartered) and all the other analytes was calculated as follows:

$$u(x) = \text{Concentration } (x) \text{ x Relative standard uncertainty } \frac{u(x)}{x}$$

$$= 14.40 \text{ mg. kg}^{-1} \text{ x } 4.02\%$$

$$= \underline{0.58 \text{ mg. kg}^{-1}}$$

Multiplying this uncertainty with a coverage factor of 2 (i.e. $k = 2$) for approximately 95% confidence level, the uncertainty then becomes 14.40 ± 1.16 mg. kg^{-1}, see table 29 to 32 for the rest of the analytes in all the different sampling techniques used.

(i) Sieved & Ground, Coned and Quartered Samples

Table 29: Reporting the expanded uncertainties for sieved and ground, cone and quartered samples

Sample	As (mg. kg^{-1})	Cd (mg. kg^{-1})	Cr (mg. kg^{-1})	Pb (mg. kg^{-1})
1	14.40 ± 1.16	19.91 ± 0.45	15.39 ± 0.94	30.68 ± 0.82
2	13.59 ± 1.09	20.22 ± 0.46	15.98 ± 0.98	31.12 ± 0.83
3	14.94 ± 1.20	19.67 ± 0.44	16.06 ± 0.99	31.31 ± 0.84
4	14.64 ± 1.18	19.91 ± 0.45	15.06 ± 0.92	30.40 ± 0.81

Looking at the expanded uncertainties of the coning & quartering sub-sampling technique discussed above as well as the relative standard errors, it appears that there were more errors in the determination of the arsenic and chromium. The low %RSD for the cadmium and lead showed that less errors were associated with the determination of these two elements.

(ii) Sieved & Ground, Random Samples

Table 30: Reporting the expanded uncertainties for sieved and ground random samples

Sample	As (mg. kg^{-1})	Cd (mg. kg^{-1})	Cr (mg. kg^{-1})	Pb (mg. kg^{-1})
1	14.28 ± 0.34	19.94 ± 0.46	15.94 ± 0.66	30.52 ± 0.21
2	14.32 ± 0.34	20.22 ± 0.47	15.64 ± 0.65	30.76 ± 0.22
3	14.54 ± 0.35	19.66 ± 0.45	15.85 ± 0.66	30.71 ± 0.21
4	14.64 ± 0.35	19.92 ± 0.46	15.21 ± 0.63	30.61 ± 0.21

The expanded uncertainties were lower for sieved & ground random samples compared to the coning & quartering. These findings suggested that this technique was the most appropriate to use since less errors were produced. The F-test performed to measure precision showed that it was only the arsenic and lead where the two subsampling techniques differed significantly. However, the precisions were close and had the 97% confidence been used instead of 95% there would have been no difference.

(iii) Non-sieved & Non-ground

Table 31: Reporting the expanded uncertainties for sieved and ground coned and quartered samples

Sample	As (mg. kg^{-1})	Cd (mg. kg^{-1})	Cr (mg. kg^{-1})	Pb (mg. kg^{-1})
1	14.36 ± 0.99	25.92 ± 5.38	19.11 ± 2.52	47.82 ± 1.99
2	15.38 ± 1.06	25.97 ± 5.39	19.44 ± 2.57	50.28 ± 2.09
3	14.93 ± 1.02	20.62 ± 4.28	19.73 ± 2.60	49.05 ± 2.04
4	13.45 ± 0.92	24.57 ± 5.10	22.03 ± 2.91	49.43 ± 2.06

Poor precision was noted for the concentrations of each analyte in the subsamples and especially for cadmium & chromium. The importance of sample preparation in order to obtain reproducible measurements with a higher certainty was demonstrated. To conclude on all the work done on the ICP-OES system, it can be said that the uncertainty arising from the sub-sampling techniques used was the biggest contributor to the overall uncertainty in the concentrations of the analytes when the modelling approach of uncertainty estimation was used.

3.9 Selection of the best method for cadmium analysis

The repeatability of the cadmium measurement in the ICP-OES had the highest relative standard uncertainty of 0.88% compared to the other analytes. As a result the concentration of this analyte varied the most in the different subsampling techniques used. The presence of an interferent can be seen in the spectrum below. A large peak appears at a slightly lower wavelength and masks the Cd line at 226.502 nm. This interferent (although not a direct overlap) significantly contributes to the Cd signal and thus makes the use of this line impossible.

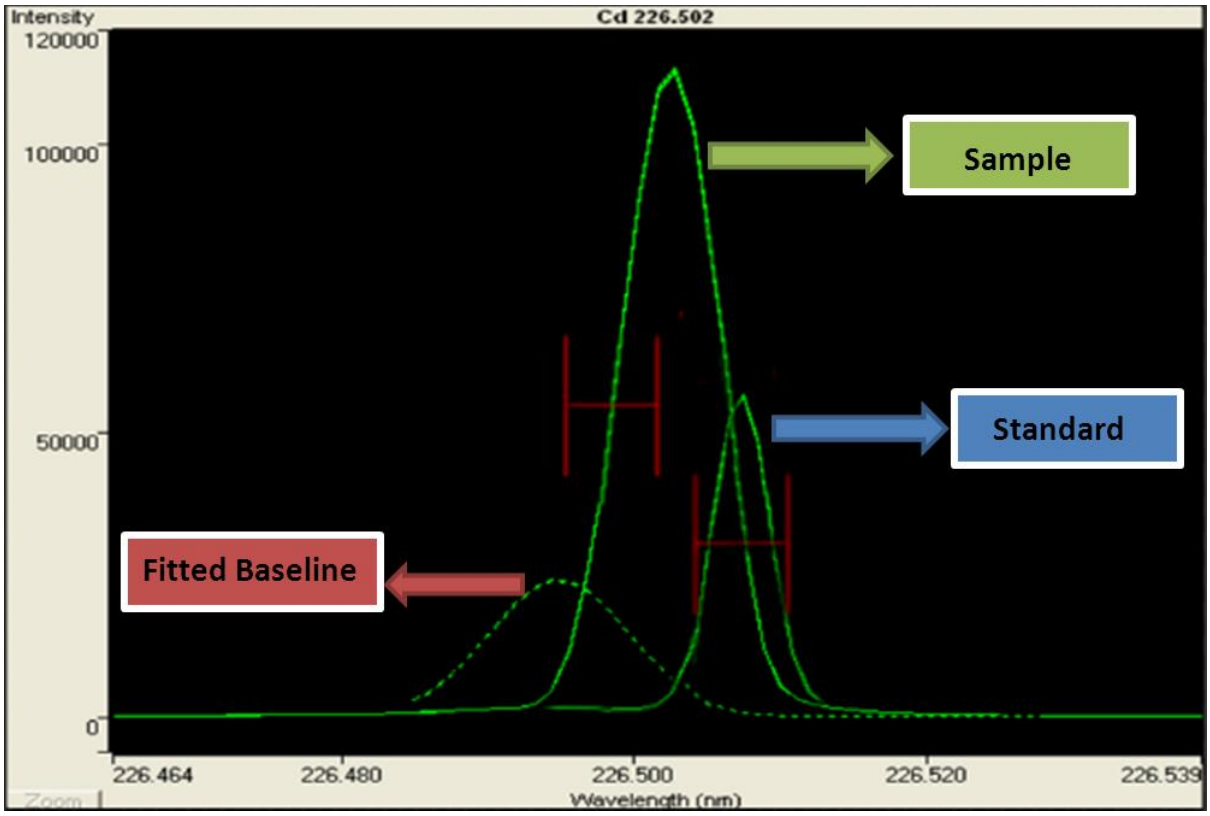


Figure 32: The interference observed in the determination of the cadmium

Moreover, the actual matrix of the sample was also suspected on being a potential cause of this lack of repeatability. If there is a presence of an interferent in the matrix of the sample and it is able to mask the cadmium signal either partially or completely, then a lack of reproducibility would be expected as a function of varying concentration of the interferent. It was for this reason that other alternatives for the determination of the concentration of the cadmium (i.e. using Graphite Furnace) had to be investigated. Another alternative would be to use a different emission line of the cadmium on the ICP-OES, however it was stated in Chapter 2 why the other alternative emission lines could not be used.

3.9.1 The determination of cadmium using the graphite furnace

An alternative technique for measuring the cadmium was used and this was the graphite furnace atomic absorption spectroscopy. The same sample preparation procedures used for the ICP-OES work were also used for this instrument. The sources of uncertainty were the same to those of the ICP-OES and their contributions were also used for the GFAAS as well.

3.9.2 GFAAS reproducibility and contribution to uncertainty budget

A repeatability experiment was performed to determine the reproducibility of the GFAAS in measuring the concentration of the cadmium. This was done by measuring a 10 µg. L^{-1} cadmium standard solution three times using the instrument operating conditions listed in section 2.5.3. The concentration of this standard solution was chosen arbitrarily and the standard deviation resulting from these repeat measurements was used to calculate the relative standard uncertainty, see table 32.

Table 32: The reproducibility experiment of the cadmium using a 10 µg. L^{-1} standard

Description	Value (x) (µg. L^{-1})	Standard uncertainty, $u(x)$ (µg. L^{-1})	Relative standard uncertainty, $\frac{u(x)}{x}$ %
Repeatability of cadmium, R_{Cd}	9.450	0.0057	0.60
	9.461		
	9.458		

Although the average concentration of the standard solution was 9.46 µg. L^{-1} and not exactly 10 µg. L^{-1}, the reproducibility of the cadmium was marginally improved on the GFAAS as can be seen from a low %RSD of 0.60% compared to the 0.88% obtained for the ICP-OES.

3.9.3 The F-test to compare the difference between the ICP-OES & GFAAS reproducibility

The difference between the precision of the ICP-OES and GFAAS for the determination of cadmium was compared with the F-test using 3 degrees of freedom for both the numerator and denominator, the results of the test are shown in table 33.

Table 33: The F-test to compare the precisions of the ICP-OES & GFAAS for determining cadmium

Instrument	Relative standard uncertainty, $\frac{u(x)}{x}$ %	F-Test		
		F	F_{crit}	P_{value}
ICP-OES	0.88	2.15	9.28	0.27
GFAAS	0.60			

The difference between the reproducibility of the instruments was found to be insignificant as the probability value was calculated to be > 0.05. Nevertheless, the %RSD of the GFAAS reproducibility was lower than that of the ICP-OES and this made this instrument to be eligible for cadmium determination. The low %RSD of the GFAAS was brought about by the use of an optimised furnace program of 900/1400 ^{o}C thermal pyrolysis & atomization temperatures.

3.9.4 GFAAS determination of the cadmium before optimization

In Chapter 2 section 2.5.4, the calibration curve of the graphite furnace before optimization of the furnace program was shown. The calibration curve had a good correlation coefficient of 0.911 and therefore since the standards were measured successfully, at that stage there was no reason to expect the samples to behave differently. Table 34 below shows the cadmium concentrations measured in the four digested random subsamples before optimization. The standard deviations of the concentrations obtained from these random subsamples were used in the estimation of the relative standard uncertainties.

Table 34: Cadmium concentrations of the random samples before optimization

Sample	Cd (mg. kg^{-1})	Standard uncertainty, (mg. kg^{-1})	Relative standard uncertainty, $\frac{u(x)}{x}$ %
1	4.99	2.01	73.6
2	3.85		
3	1.26		
4	0.83		

The relative error was very high for these concentrations and several repeat determinations were performed and no improvement was seen in reducing the relative error. The instrument default furnace program (i.e. 700 ^{o}C for thermal pyrolysis and 1400 ^{o}C for atomization) was therefore not suitable for determining the cadmium in the matrix of the samples. The optimization of the furnace program was discussed in section 2.5.5 of Chapter 2 where the instrument default furnace program was modified and it was discovered that a thermal pyrolysis temperature of 900 ^{o}C and an atomization temperature of 1400 ^{o}C were appropriate for the determination of the cadmium.

3.9.5 Determination of Cd using the optimized furnace program

When the furnace program was optimized, freshly digested sieved & ground random samples were analysed for the presence of the cadmium and the concentrations measured are shown in table 35 below. The calibration curve for the cadmium standard solution was shown in section 2.5.6. The linearity of the calibration curve was improved and this was indicated by an improved correlation coefficient.

Table 35: Cadmium concentrations of the random samples after a new furnace program

Sample	Cd ($mg.\ kg^{-1}$)	Standard uncertainty, ($mg.\ kg^{-1}$)	Relative standard uncertainty, $\frac{u(x)}{x}$ %
1	6.25	0.95	14.44
2	6.65		
3	7.89		
4	5.63		

The reproducibility improved and the relative standard error was lowered from 73.6% to 14.4%. Performing the F-test on the two relative standard errors for before and after furnace program optimization showed that the difference between the relative errors was significant as the probability value calculated to be 0.01 was lower than 0.05 at 95% confidence level. It should be noted that the relative error for the cadmium determination on the ICP-OES was 1.15% and this was lower than the 14.4% obtained for the GFAAS. Therefore according to these findings, the graphite furnace was unsuccessful in improving the error (precision) associated with the determination of the cadmium in the digested sewage sludge subsamples. The GFAAS results show a significantly higher uncertainty than that obtained by the ICP-OES. However, the accuracy of the ICP-OES analysis was in question because of a large overlapping peak in the spectrum due to an unidentified species. This could have been verified by spiking or with the use of a CRM which was not available at the time. However, this was not done as the overlapping peaks could not be resolved to allow proper integration of the Cd peak. Therefore, although the precision by GFAAS was poorer for the samples it is possible that it may in fact be more accurate compared to the ICP-OES. Therefore, because of the likely problems in accuracy by ICP-OES it was decided to use the less precise GFAAS method for analysis of Cd.

3.9.6 The overall uncertainty in the determination of cadmium was calculated using equation 6 as follows:

$$u(x) = \text{Concentration } (x) \text{ x Relative standard uncertainty } \frac{u(x)}{x}$$

$$= 6.61 \text{ mg. kg}^{-1} \text{ x } 14.4\%$$

$$= \underline{0.95 \text{ mg. kg}^{-1}}$$

Multiplying this uncertainty with a coverage factor of 2 (i.e. $k = 2$) for approximately 95% confidence level, the uncertainty then becomes 6.61 ± 1.90 mg. kg^{-1}. As mentioned already, the degree of accuracy for this estimated uncertainty cannot be commented on due to the lack of availability of a certified reference material. The overall uncertainty of the cadmium concentration was higher in comparison to the ICP-OES and these findings were contrary to what was expected. Further investigations need to be carried out to develop a method suitable for the determination of cadmium with minimum errors.

3.10 Determination of mercury in the VGA-77 AAS system

The sources of uncertainty associated with the determination of the concentration of mercury using the VGA-77 AAS system were similar to those of the ICP-OES and therefore the same cause & effect diagram was applicable.

3.10.1 VGA-77 AAS reproducibility and contribution to uncertainty budget

An experiment was performed to determine the repeatability of the concentrations of mercury in the VGA-77 AAS system. This was done by measuring an arbitrarily chosen 3 µg. L^{-1} mercury standard solution three times on the instrument. Using the absorbance readings in table 36 below together with the calibration curve shown in section 2.6.2, the concentrations were calculated. The standard uncertainty as well as the relative standard uncertainty was calculated for the concentrations and the results are shown in table 36 below.

Table 36: The repeatability experiment to determine the standard uncertainty of the mercury

Description	Absorbance	Value (x) (µg. L^{-1})	Standard uncertainty, $u(x)$ (µg. L^{-1})	Relative standard uncertainty, $\frac{u(x)}{x}$ %
Repeatability of mercury, R_{Hg}	0.035	3.275	0.083	2.61
	0.033	3.108		
	0.034	3.192		

The calculated relative error was found to be low and since the mercury was not measured in the ICP-OES and/or GFAAS, there were no means of comparing the precision of the VGA-77 AAS with these two instruments.

3.10.2 Quantifying the uncertainty in the random subsampling for the VGA-77 AAS

It has been mentioned previously that for the ICP-OES either the coning & quartering or random sampling of the sieved & ground samples was appropriate to use because less relative errors were obtained. Therefore the same convention was applied for the VGA-77 AAS system and the sieved & ground random subsamples were used for the determination of mercury, see table 37 below.

Table 37: Mercury concentrations obtained from the random sampling for the VGA-77 AAS

Sample	Hg (mg. kg^{-1})	Standard uncertainty, (mg. kg^{-1})	Relative standard uncertainty, $\frac{u(x)}{x}$ %
1	0.181	0.0046	2.56
2	0.183		
3	0.186		
4	0.175		

3.10.3 Comparing the repeatability of the VGA-77 AAS against random subsamples

The precision of the VGA-77 AAS in determining the repeatability was compared to its precision of measuring the concentration of mercury in the sieved & ground random subsamples using the F-test, the results are shown in table 38.

Table 38: The F-test to compare the precisions of the VGA-77 AAS against random subsamples

Instrument	Relative standard uncertainty, $\frac{u(x)}{x}$ %	F-Test		
		F	F_{crit}	P_{value}
VGA-77 AAS repeatability	2.61	1.03	9.28	0.49
Random subsamples	2.56			

There was no significant difference between the VGA-77 AAS repeatability against the random subsamples as shown by a probability value of 0.49. The relative standard uncertainty was used in the calculation of the overall uncertainty in the same manner as it was for the ICP-OES as well as the GFAAS.

3.10.4 The overall uncertainty of the mercury concentration using the VGA-77 AAS system

$$u(x) = \text{Concentration } (x) \text{ x Relative standard uncertainty } \frac{u(x)}{x}$$

$$= 0.181 \text{ mg. kg}^{-1} \text{ x } 2.56\%$$

$$= \underline{0.00463 \text{ mg. kg}^{-1}}$$

Multiplying this uncertainty with a coverage factor of 2 (i.e. $k = 2$) for approximately 95% confidence level, the uncertainty then becomes 0.181 ± 0.0093 mg. kg^{-1}. The degree of accuracy for this estimated uncertainty cannot be commented on due to the lack of availability of a certified reference material. The investigation was further expanded into assessing the effect of nitric in the mercury concentrations measured using the VGA-77 AAS. The rationale for performing this assessment is given in section 3.10.5.

3.10.5 The effect of nitric acid concentration in the determination of mercury

There are many processes that occur in the cold vapour technique for the determination of mercury and these processes can be affected by numerous factors. One of these factors is the concentration of the acid used in the digestion (Shrader & Hobbins, 2010). In an investigation to assess whether the concentration of the HNO_3 had any effect in the mercury determination, the calibration standard solutions were matrix matched with an approximately similar concentration of the HNO_3 as in the digested sewage sludge samples. The calibration curve shown in section 2.6.3 had a high correlation coefficient of 0.9907 and a slope of 0.0121 and these were very close to the 0.9961 & 0.0120 shown in section 2.6.2 for the correlation coefficient and slope respectively of the non-matrix matched standard solutions.

Table 39 below shows the mercury concentrations calculated from the section 2.6.3 calibration curve together with the standard uncertainty as well as the relative standard uncertainty.

Table 39: Mercury concentration in the random subsamples for matrix matched calibration of the VGA 77 – AAS

Sample	Hg (mg. kg^{-1})	Standard uncertainty, (mg. kg^{-1})	Relative standard uncertainty, $\frac{u(x)}{x}$ %
1	0.201	0.00399	1.94
2	0.195		
3	0.203		
4	0.196		

Comparing the precisions between water based and nitric acid based standards showed no significant difference when the F-test was performed, see table 40. However, the t-test showed that there has been a significant increase in concentration (~10%). Therefore matrix matching did not have a direct effect on precision but did have an effect on the accuracy of the analyses. Therefore if the nitric acid content was variable in the analysis then this would have an effect on the precision of the analysis.

Table 40: The F-test to compare the precisions when the effect of nitric acid is assessed

Instrument	Relative standard uncertainty, $\frac{u(x)}{x}$ %	F-Test		
		F	F_{crit}	P_{value}
Water based Standard Solutions for Calibration	2.56	1.74	9.28	0.13
Nitric acid matched Standard Solutions for Calibration	1.94			

The t-test showed that although the concentrations may have increased slightly for matrix matched samples, there was no difference in the precision. Nevertheless it is recommended for future determinations of mercury by VGA-77 AAS that the standard solutions must be matrix matched with the same concentration of nitric acid as in the samples to improve the accuracy. The use of CRM is also highly recommended for the confirmation of the accuracy in future determinations of mercury using the VGA-77 AAS detection system.

3.10.6 The overall uncertainty of the mercury concentration using the VGA-77 AAS system for matrix matching:

$$u(x) = \text{Concentration } (x) \text{ x Relative standard uncertainty } \frac{u(x)}{x}$$

$$= 0.199 \text{ mg. kg}^{-1} \text{ x } 1.94\%$$

$$= \underline{0.00386 \text{ mg. kg}^{-1}}$$

Multiplying this uncertainty with a coverage factor of 2 (i.e. $k = 2$) for approximately 95% confidence level, the uncertainty then becomes 0.199 ± 0.0077 mg. kg^{-1}. The overall uncertainty decreased due to the effect of matrix matching the standard solutions with same nitric acid as the digested samples.

3.11 Determination of mercury in the VGA-77 ICP-OES system

The VGA-77 ICP-OES system was investigated as an alternative to the VGA-77 AAS system for the determination of mercury. The primary objective was to see if any improvements in precision could be gained by using a more sensitive detection method. The sources of uncertainty associated with the determination of the concentration of the mercury in the VGA-77 ICP-OES system were similar to those of all the instruments already discussed and therefore the same cause & effect diagram was applicable.

3.11.1 VGA-77 ICP-OES reproducibility and contribution to uncertainty budget

A repeatability experiment was performed to determine the repeatability of the concentrations of the mercury in the VGA-77 ICP-OES system. This was done by measuring an arbitrarily chosen 6 µg. L^{-1} mercury standard solution three times on the instrument. The standard uncertainty as well as the relative standard uncertainty were calculated for the concentrations and the results are shown in table 41 below.

Table 41: The repeatability experiment to determine the standard uncertainty of the mercury

Description	Value (x) (µg. L^{-1})	Standard uncertainty, $u(x)$ (µg. L^{-1})	Relative standard uncertainty, $\frac{u(x)}{x}$ %
Repeatability of mercury, R_{Hg}	5.878	0.010	0.172
	5.898		
	5.892		

The relative standard error for the reproducibility of the standard solutions was significantly different from that obtained for the VGA-77 AAS system (i.e. probability value of 0.0005), see table 42.

Table 42: The F-test to compare the precision of the VGA-77 AAS against VGA-77 ICP-OES

Instrument	Relative standard uncertainty, $\frac{u(x)}{x}$ %	F-Test		
		F	F_{crit}	P_{value}
VGA-77 AAS repeatability	2.61	230	9.28	0.0005
VGA-77 ICP-OES repeatability	0.17			

It is expected that the VGA-77 ICP-OES system should produce better precision as it is a more sensitive detection system and hence relative errors for low concentrations would be expected to be lower.

3.11.2 Quantifying the uncertainty in the random subsampling for the VGA-77 ICP-OES

Sieved & ground random subsamples were used for the determination of the mercury using the VGA-77 ICP-OES system. The standard uncertainty as well as the relative standard uncertainty calculated are reported in table 43.

Table 43: Mercury concentrations obtained from the random sampling for the VGA-77 ICP-OES

Sample	Hg (mg. kg^{-1})	Standard uncertainty, (mg. kg^{-1})	Relative standard uncertainty, $\frac{u(x)}{x}$ %
1	0.151	0.0030	1.98
2	0.154		
3	0.149		
4	0.147		

It was noted that the precision for the samples was poorer than for standards (see F-test results in table 44) and this probably reflected the level of heterogeneity in the samples. It was further noted that the concentration of the mercury in the samples analysed by the VGA-77 ICP-OES system were lower than in the

AAS system. It remained unclear why this was the case and it highlighted the need for a CRM to be used to validate the accuracy of the results.

3.11.3 Comparing the VGA-77 ICP-OES repeatability precision against random subsamples

The precision of the VGA-77 ICP-OES in determining the repeatability was compared to its precision of measuring the concentration of mercury in the sieved & ground random subsamples using the F-test, the results are shown in table 44 below.

Table 44: The F-test to compare the precisions of the VGA-77 ICP-OES against random subsamples

Instrument	Relative standard uncertainty, $\frac{u(x)}{x}$ %	F-Test		
		F	F_{crit}	P_{value}
VGA 77 – ICPOES repeatability	0.17	132	9.28	0.0011
Random sampling	1.98			

Since it was noted that matching the nitric acid matrix was important in the VGA-77 AAS measurements; analyses using the VGA-77 ICP-OES system was calibrated with matrix matched standards. The relative error (1.98%) obtained for the random subsamples using the VGA-77 ICP-OES showed no significant difference to the "matrix matched" random subsamples of the VGA-77 AAS (1.94%) in section 6.5 (F-test gave a value of *p* = 0.49). This lack of difference between techniques probably reflected the level of homogeneity of the sample itself. The difference in actual concentration highlighted the need for an appropriate CRM to be used.

3.11.4 The overall uncertainty of the mercury concentration using the VGA-77 ICP-OES system was calculated as follows:

$$u(x) = \text{Concentration } (x) \text{ x Relative standard uncertainty } \frac{u(x)}{x}$$

$$= 0.150 \text{ mg. kg}^{-1} \text{ x } 1.98\%$$

$$= \underline{0.00297 \text{ mg. kg}^{-1}}$$

Multiplying this uncertainty with a coverage factor of 2 (i.e. $k = 2$) for approximately 95% confidence level, the uncertainty then becomes 0.150 ± 0.0059 mg. kg^{-1}. This overall uncertainty was lower than that of the VGA-77 AAS for the "matrix matched" samples. These findings suggested that the VGA-77 ICP-OES was the most appropriate method to use for the determination of the mercury for highest precision.

3.11.5 Mercury recovery on the VGA-77 AAS against the VGA-77 ICPOES system

The comparison of the VGA-77 AAS system with the VGA-77 ICP-OES system was expanded by a "Matrix Spiking" experiment which is a technique used for the evaluation of the performance of analytical procedure when testing a specific sample (matrix) type (Thermoscientific, 2011). The matrix spike test helps in answering the question of whether we are getting valid results when a particular method is used for testing a sample. A high recovery of the spike increases the confidence in the accuracy and validity of the sample test results (Thermoscientific, 2011).

For both detection techniques, the spiking experiments were described in sections 2.6.4 and 2.7.3 for the VGA-77 AAS and VGA-77 ICP-OES systems respectively. It was mentioned that for both detection techniques, the digested samples were analysed first to determine the mass of mercury present initially and then spiked with 0.025 µg of mercury through the use of a standard solution and then re-measured, see table 45 below for the results of this recovery experiment.

Table 45: The recovery experiment for the mercury on the VGA-77 AAS system

Masses of the mercury (µg)			% Recovery	% Average Recovery
Initial Mass	Added Mass	Final Mass		
0.1320	0.025	0.1520	80.0	60.1
0.1440	0.025	0.1540	40.0	
0.1315	0.025	0.1421	42.4	
0.1298	0.025	0.1493	78.0	

There was no consistency in the recovery of the additional mercury in the samples determined with the VGA-77 AAS detection technique, however all were significantly less than 100% recovery. This level of inconsistency was confusing and it did not correlate to the reproducibility experiments. It showed that the results possibly had other issues (e.g. the spiked mercury was getting chelated or bound to other species and as a result could not be analysed) besides simple analytical reproducibility. These findings meant that the analytical procedure was not working well for the samples as the matrix spike results were much lower than expected.

Contrary to the VGA-77 AAS detection system, the recovery of the mercury spike in the digested samples was close to 100% with the VGA-77 ICP-OES detection system. These good recoveries (see table 46) displayed a higher degree of precision and that the accuracy was okay. It was through these findings that the VGA-77 ICP-OES detection system was preferred for the analysis of mercury in the sewage sludge samples.

Table 46: The recovery experiment for the mercury on the VGA-77 ICP – OES system

Masses of the mercury (µg)			% Recovery	% Average Recovery
Initial Mass	Added Mass	Final Mass		
0.1549	0.025	0.1782	93.2	94.3
0.1169	0.025	0.1410	96.4	
0.1092	0.025	0.1322	92.0	
0.1128	0.025	0.1367	95.6	

3.12 Overall summary of errors and comparison with literature data

The cause & effect diagram helped in showing what the major contributions were to the overall uncertainty in the measurements. Using the described sampling techniques, digestion and analytical methods, analyses were performed which allowed the errors to be reduced to levels that permitted for the sludge to be placed into different categories according to the levels listed in the "South African Sludge Management Guidelines" assuming no bias in the results. Contributions to the overall uncertainty by the different parts of the procedure are summarized herein for each element analysed.

(i) Arsenic

The arsenic was determined by ICP-OES only and the overall uncertainty was quantified in terms of precision. Shown below in table 47 below are all the relative errors for each part of the procedure followed. The uncertainty based on accuracy can only be dealt with by CRM analysis which was not available at the time the research was conducted. Assuming no systematic errors, the data obtained gives an understanding of the uncertainty expected in terms of precision.

Table 47: Errors associated with each part of the procedure for the determination of arsenic

Main Cause	**Sub-cause**	**Total error (% RSD)**
		ICP-OES
Solids content	Error in weighing	0.0047
Acid Digestion	Volume of acid taken & Acid purity	
Calibration	Volume of the volumetric flask	0.073
Instrument	Repeatability	0.87
Sampling	Sieving & Grinding: Coning & Quartering	4.02
	Sieving & Grinding: Random Sampling	1.2
	Non-sieved & Non-ground	3.43

The precision in the determination of the solids content had a small contribution to the overall uncertainty and this finding was the same for the other elements in all detection systems used. The uncertainty arising from the precision of the volume of the volumetric flask for the calibration was also very small and its contribution was deemed to be negligible. Based on the repeatability experiment for the standard solution, the relative error of the repeat measurements was low (i.e. 0.87%) and this suggested that a high precision was attained. This finding was further supported by comparing the repeatability precision to that of the digested sewage sludge samples (%RSD = 1.2) which showed that the difference was not significant.

The overall assessment of the contributions to the overall uncertainty of the concentration for this element showed that the biggest contribution was from the "Sampling" technique. Moreover, of all the different sample preparation techniques used, the random subsampling of the sieved and ground samples produced less errors (i.e. higher precision) for the analysis of the arsenic. Lastly, the concentration and overall expanded uncertainty of the arsenic in the sewage sludge samples was found to be 14.40 ± 0.35 mg. kg^{-1}. This concentration was within the tolerable limit to that given in the "South African Sludge Management Guidelines" study by Snyman *et al.,* 2006. The guideline uses three classification systems; Microbial class, Stability class and Pollutant class which is of interest to this research as it deals with the metal concentrations, see tables 48 & 49 below.

Table 48: The South African wastewater sludge classification system (Snyman & Herselman, 2006)

Classification	**Best quality**	**Intermediate quality**	**Worse quality**
Microbiological class	A	B	C
Stability class	1	2	3
Pollutant class	a	b	C

In the study by Snyman *et al.,* 2006, the metals were extracted from the wastewater sludge using the aqua regia digestion ISO 11466 method and the analysis was performed with the ICP-AES system. Therefore, based on the values given in the table above the value of 14.40 ± 0.35 mg. kg^{-1} determined in this study can be classified as "best quality". Although we remain uncertain about the accuracy of this concentration since no CRMs were used, the amount of systematic error would have to be very high to move it out of the "best quality" classification. Therefore this gives us confidence that the level analysed is probably realistic.

Table 49: Metal limits for South African Wastewater sludges (Snyman & Herselman, 2006)

Element	Metal Limits for each Pollutant Class (mg. kg^{-1})		
	a	**b**	**c**
As	<40	40 – 75	>75
Cd	<40	40 – 85	>85
Cr	<1200	1200 – 1300	>3000
Pb	<300	300 – 840	>840
Hg	<15	15 – 55	>55

Moreover, a recent study by Mdlambuzi *et al.,* 2014 at Umgeni Darvill Waste Water Works (origin of this sewage sludge) for the investigation of the spatial and vertical distribution of heavy metals and their availability in soils showed that the concentration of the arsenic in the different soil depths was in the range of 0.002 – 15.991 mg. kg^{-1} by aqua regia digestion as well as ICP-OES analysis. The concentration range was reported with no level of precision or degree of accuracy but nevertheless, the concentration determined in this study fell within this range.

To broaden the comparisons further, a study conducted by Eriksson, 2001 for analysing the concentration of 61 trace elements in sewage sludge in Sweden showed that the mean concentration was 4.70 mg. kg^{-1} (the range was 1.6 – 14 mg. kg^{-1}). The standard deviation for this analysis was 2.60 mg. kg^{-1} and an RSD of 55%. The precision of this determination was lower than the 1.20% found in this research. The range of expected values for the arsenic concentration can be quite high e.g. a study by Tiruneh *et al.,* 2014 in seven different wastewater treatment plants in Swaziland for the evaluation of the risk of heavy metals in sewage sludge intended for agricultural application showed a range of 5 – 139 mg. kg^{-1} for the Piggs Peak wastewater plant, see table 50 below.

Table 50: Results of arsenic analysis in sewage sludge found in literature

Element	Metal concentration in sewage sludge (mg. kg^{-1})	
	Eriksson, 2001 (Sweden)	Tiruneh *et al.,* 2014 (Swaziland)
As	1.60 – 14	5 – 139

(ii) Cadmium

The cadmium was first analysed by the ICP-OES and the contributions to the overall uncertainty by the solids content and the acid digestion steps were the same as all the elements (see table 51) because one sample was analysed for the presence of different metal pollutants. The relative error of the repeat measurements was low (i.e. 0.88%) and it was similar to that of the arsenic. Moreover, the difference between the precision of the repeatability experiment of the standard solution and the sewage sludge samples was shown to be insignificant at 95% confidence level.

Table 51: Errors associated with each part of the procedure in the determination of the cadmium

Main Cause	Sub-cause	Total error (% RSD)	
		ICP-OES	GFAAS
Solids content	Error in weighing	0.0047	0.0047
Acid Digestion	Volume of acid taken & Acid purity		
Calibration	Volume of the volumetric flask	0.073	0.073
Instrument	Repeatability	0.88	0.60
Sampling	Sieving & Grinding: Coning & Quartering	1.13	
	Sieving & Grinding: Random Sampling	1.15	14.44
	Non-sieved & Non-ground	10.38	

The precision of the sampling techniques i.e. coning & quartering and random sampling was not significantly different and therefore their contribution to the overall uncertainty was the same. The precision of the non-sieved and non-ground subsamples was significantly different from the two other sampling strategies and these findings demonstrated how pivotal the sample pre-treatment step was in reducing errors. It was at this stage where it was concluded that any of these two sampling techniques were appropriate for the analysis of cadmium. The cadmium concentration was found to be 19.94 ± 0.46 mg. kg^{-1} and this meant that the sewage sludge may be classified as best quality according to the South African Sludge Management Guidelines which showed a value of <40 mg. kg^{-1}. Furthermore, the concentration of the cadmium in a study by Mdlambuzi *et al.,* 2014 was in the range of 0.217 – 19.814 mg. kg^{-1} by ICP-OES analysis and the concentration of 19.94 ± 0.46 mg. kg^{-1} obtained in our research fell within the range. However, the accuracy of this determination was in question due to a spectral line which was seen to interfere with the cadmium line and had the CRM been available at the time, this ambiguity would have been resolved. These findings led into developing a new method for the analysis of cadmium and this was the GFAAS. This detection method was expected to improve the accuracy of the analysis of the cadmium, however the potential improved accuracy came at a cost in terms of precision compared to the ICP-OES.

The cadmium levels found in our study were slightly higher than those reported in the Limpopo and Sweden studies, see table 52 below. However, the ranges of cadmium can also appear to be large as it can be seen in the study by Sterritt *et al.,* 1980 for the concentrations of heavy metals in forty sewage sludges in England which had 1.54 – 110 mg. kg^{-1} of which our determined values fell in the bottom of. In a publication by Eriksson, 2001 the mean concentration of the cadmium using ICP-AES for analysis was found to be 1.4 mg. kg^{-1} with a standard deviation of 1.5 mg. kg^{-1}. Given the standard deviation, the precision was calculated as the %RSD = 107 indicating that a large variation in the metal content concentration can be expected depending on the site measured.

Table 52: Result of cadmium analysis in sewage sludge samples found in literature

Element	**Metal concentration in sewage sludge (mg. kg^{-1})**		
	Sterritt *et al.,* 1980 (England)	Eriksson, 2001 (Sweden)	Shamuyarira *et al.,* 2013 (South Africa – Limpopo)
Cd	1.54 – 110	0.58 – 11	0.32 – 3.10

Although in the most recent study by Shamuyarira *et al.,* 2013 a very narrow range was shown of which the determined concentration in our research did not fall in and even though the estimated uncertainty was higher for the GFAAS compared to the ICP-OES, the interference seen with the ICP-OES led into making a final decision of using a less precise GFAAS for analysing cadmium but hopefully more accurate.

(iii) Chromium

The analysis of the chromium was also performed in the ICP-OES (see table 53) and comparing the precision of the repeatability experiment of the standard solution with the sewage sludge concentration showed that there was a significant difference at 95% confidence level.

Table 53: Errors associated with each "cause" for the determination of chromium in the ICP-OES

Main Cause	**Sub-cause**	**Total error (% RSD)**
Solids content	Error in weighing	0.0047
Acid Digestion	Volume of acid taken & Acid purity	
Calibration	Volume of the volumetric flask	0.073
Instrument	Repeatability	0.68
Sampling	Sieving & Grinding: Coning & Quartering	3.07
	Sieving & Grinding: Random Sampling	2.08
	Non-sieved & Non-ground	6.60

The precision in the analysis of the samples was higher for the sieved & ground random samples as shown by the low %RSD. These findings show how important a contribution the sampling step is to the overall uncertainty budget. The concentration of the chromium was found to be 15.66 ± 0.64 mg. kg^{-1}. According to the metal limit guideline table, this concentration of the chromium qualified the sewage sludge to be of best quality. This chromium concentration fell out of the 39 – 1841 mg. kg^{-1} range shown in the study by Mdlambuzi *et al.,* 2014.

Table 54: Result of chromium analysis in sewage sludge samples found in literature

Element	Metal concentration in sewage sludge (mg. kg^{-1})			
	Sterritt *et al.,* 1980 (England)	Eriksson, 2001 (Sweden)	Shamuyarira *et al.,* 2013 (South Africa – Limpopo)	Tiruneh *et al.,* 2014 (Swaziland)
Cr	57 – 5190	10 – 83	35 – 134	409 – 648

However Eriksson, 2012 had a range of 10 – 83 mg. kg^{-1} (see table 54 above) which covered the concentration determined in this research. The mean chromium concentration in Eriksson's publication was 33 mg. kg^{-1} with a standard deviation of 16 mg. kg^{-1}.

(iv) Lead

Comparing the precision of the repeatability experiments of the lead standard solution with the sewage sludge concentration showed no difference at 95% confidence level. The total errors associated with each step of the procedure for analysis of lead are shown in table 55 below.

Table 55: Errors associated with each "cause" for the determination of lead in the ICP-OES

Main Cause	Sub-cause	Total error (% RSD)
Solids content	Error in weighing	0.0047
Acid Digestion	Volume of acid taken & Acid purity	
Calibration	Volume of the volumetric flask	0.073
Instrument	Repeatability	0.35
Sampling	Sieving & Grinding: Coning & Quartering	1.34
	Sieving & Grinding: Random Sampling	0.35
	Non-sieved & Non-ground	2.08

The precision in the analysis of the samples was higher for the sieved & ground random samples as shown by the low %RSD. The concentration of the lead was found to be 30.65 ± 0.21 mg. kg^{-1} and according to the metal limit guideline table, this concentration qualified the sewage sludge to be of best quality. This concentration fell within the majority of the ranges found in the literature, see table 56 below. These findings suggested that the analysis was reasonably correct. It is worth noting that the lead concentration range of 27 – 45400 mg. kg^{-1} by Sterritt *et al.,* 1980 was very high and a possible reason for this was that in 1980 petrol contained lead additives and this would be expected to contribute to the overall lead content in the sewage sludge.

Table 56: Result of lead analysis in sewage sludge samples found in literature

Element	**Metal concentration in sewage sludge (mg. kg^{-1})**			
	Sterritt *et al.,* 1980 (England)	Eriksson, 2001 (Sweden)	Shamuyarira *et al.,* 2013 (South Africa – Limpopo)	Tiruneh *et al.,* 2014 (Swaziland)
Pb	27 – 45400	11 – 110	18 – 154	7 – 89

(v) Mercury

The analysis of the mercury content in the sewage sludge was performed with two detection systems; VGA-77 AAS and VGA-77 ICP-OES as shown in table 57. These two detection systems were exclusive to this metal and findings made from the analyses are discussed herein.

Table 57: Errors associated with each "cause" for the determination of Mercury

Main Cause	Sub-cause	Total error (% RSD)	
		VGA-77 AAS	VGA-77 ICP-OES
Solids content	Error in weighing	0.0047	0.0047
Acid Digestion	Volume of acid taken & Acid purity		
Calibration	Volume of the volumetric flask	0.073	0.073
Instrument	Repeatability	2.61	0.17
Sampling	Sieving & Grinding: Random Sampling	2.56	1.98

The precision of the VGA-77 AAS in analysing the standard solution for the repeatability experiment was not different from the precision of the sewage sludge sample analysis at 95% confidence level. It was further discovered that the overall uncertainty (in terms of precision) was reduced when the calibration was performed with matrix matched standard solutions. The overall concentration of the mercury was found to be 0.181 ± 0.009 mg. kg^{-1} and comparing this with the data given in the metal limit guidelines, the sludge can be classified as best quality. The concentration range given for the mercury in the study by Mdlambuzi *et al.,* 2014 at different soil depths was 0.535 – 5.864 mg. kg^{-1} of which our concentration fell out of. Although there was no CRM available to verify the accuracy of the 0.181 ± 0.0093 mg. kg^{-1}, a spiking experiment was performed and the findings made suggested that the values obtained (100% recovery) were realistic in terms of accuracy.

In the case of the VGA-77 ICP-OES system the precision was better compared to the VGA-77 AAS. In this case there is no significant difference in precision between the repeatability measurement of a standard solution and a sewage sample. This precision of the VGA-77 ICP-OES system was better as the uncertainty was reduced i.e. 0.150 ± 0.006 mg. kg^{-1} and the concentration compared very well with the range given by Eriksson, 2001. The recoveries in the VGA-77 ICP-OES were close to 100% and these findings suggested that a better accuracy was attained with this detection system which made it the preferable system in terms of precision and accuracy for the analysis of mercury in the sewage sludge.

3.13 Predicting precision of the detection systems using the Horwitz equation

The Horwitz equation describes the relationship between the relative standard deviation (i.e. precision of the detection system) and the concentration of the analyte C (expressed as a mass fraction) as $\%\mathrm{RSD} = 2^{(1-0.5\log C)}$ regardless of the kind of analyte, matrix or method of analysis used (Lisinger *et al.,* 2006). This equation is used widely for the assessment of interlaboratory comparisons using the Horrat value. The Horrat value compares the actual precision measured with the prediction predicted by the Horwitz equation according to the equation shown below (Lisinger *et al.,* 2006).

$$\text{Horrat} = \frac{\%\mathrm{RSD}_{\text{measured}}}{\%\mathrm{RSD}_{\text{Horwitz predicted}}}$$

Equation 7: The Horrat equation for calculating the Horrat value

Deviations from the Horrat ratio on the low side (i.e. = 1 or <1) are indicative of satisfactory interlaboratory precision. Conversely, consistent deviations on the high side (i.e. >2) indicate inhomogeneity of the samples being tested and therefore a need for further method optimization (Lisinger *et al.,* 2006). For our research, the Horrat values were on the low side for all the elements in all the detection systems used. These findings meant that our precision was better than that predicted by the Horwitz equation as table 58 below shows.

Table 58: Comparing the precision of the concentrations from our research with the Horwitz prediction

Analyte	Detection Systems							
	ICP-OES		GFAAS		VGA 77 – AAS		VGA 77: ICP-OES	
	$\%RSD_{meas}$	$\%RSD_{pred.}$	$\%RSD_{meas}$	$\%RSD_{pred.}$	$\%RSD_{meas}$	$\%RSD_{pred.}$	$\%RSD_{meas}$	$\%RSD_{pred.}$
As	1.2	10.7						
Cd	1.15	10.2	14.44	12				
Cr	2.08	10.6						
Pb	0.35	9.6						
Hg					2.56	20.7	1.98	2.13

The measured %RSD was higher for the GFAAS compared to the predicted value and this was expected because it has been stated that a less precise GFAAS was used for analysing cadmium due to limitations seen with the ICP-OES. However the Horrat value was 1.2 which indicated good sample homogeneity and satisfactory precision.

CHAPTER 4

4. Conclusion

The ISO 11466 sample decomposition method is used at Umgeni Darvill Waste Water Work for the determination of metal concentration in their sewage sludge. A set of operating procedures as well as metal detection methodologies were developed & used for the analysis of the As, Cd, Cr, Pb and Hg in the sewage sludge. The use of the cause and effect diagram to identify the main sources of errors in the determination of the metal concentrations and the combination of these uncertainty components were the main aims of this research project. The cause and effect diagram met at the beginning of chapter 3 listed various contributions to the overall uncertainty of the metal concentrations analysed with the three detection systems (i.e. ICP-OES, GFAAS and VGA-77 with AAS and/or ICP-OES) is shown again in figure 33 below for referral purposes.

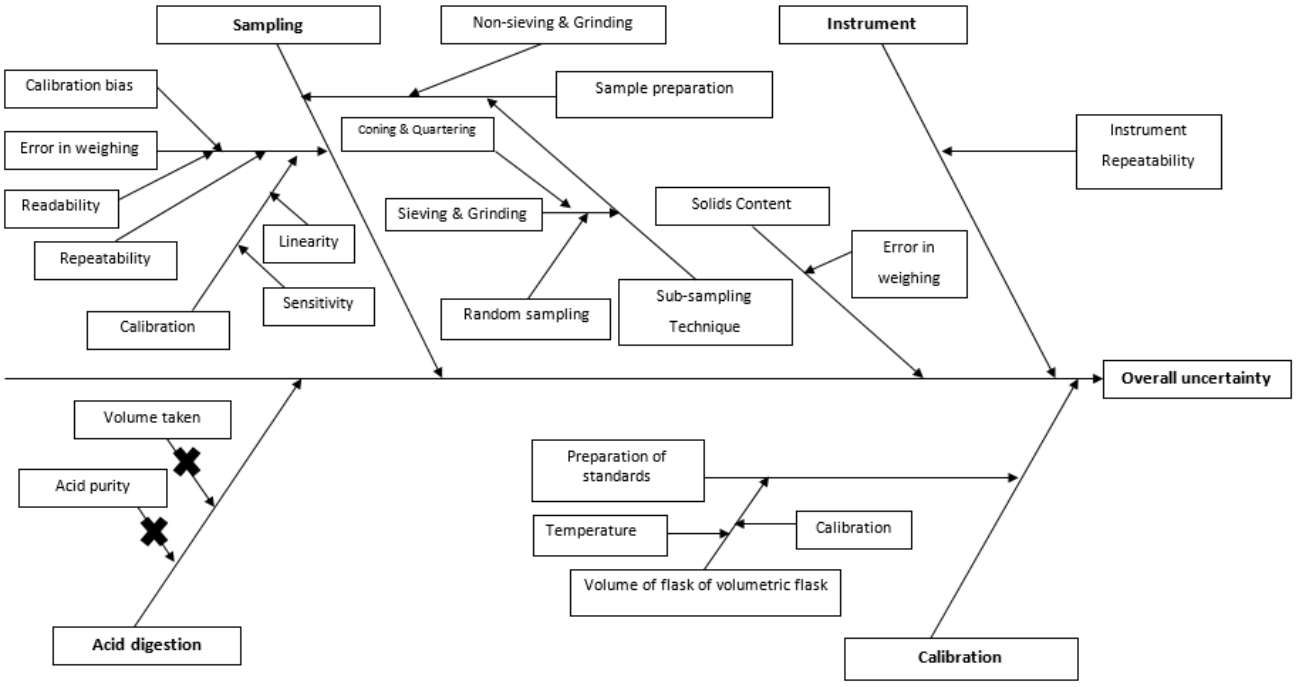


Figure 33: The cause and effect (Ishikawa) diagram for the determination of metal concentrations

Figure 33 shows the measurand or target to be the “overall uncertainty” and this overall uncertainty was determined by the considering & quantifying the contributions from the causes. Although all the branches on the cause & effect show how they contribute to the overall uncertainty, it was discovered that some of these contributions (Acid Digestion” and “Calibration”) were too small to contribute significantly to the overall uncertainty. The main causes which had significant effects and responsible for the uncertainty were “Instrument” (through the precision of the repeatability experiment) and “Sampling” (through the precision of the sub-sampling step e.g. sieving & grinding).

The ICP-OES detection method used for the analysis of the arsenic showed that the precision at which this element was determined with was high, as indicated by the low %RSD. This level of precision would unlikely be a problem when categorizing sewage sludge in terms of SA sludge management guidelines. This is because the level of the precision accounts for 2.5% of the threshold value. In other words the range of values obtained is small and would be unlikely to straddle the guideline levels, i.e. at the guideline the value would be 40 ± 1 mg. kg^{-1}. The major issue would be the accuracy of the determination of which would need the use of CRMs to fully understand the results in terms of accuracy.

The precision of the cadmium analysis in the ICP-OES was also high but the accuracy of the concentration obtained was questionable due to the interference observed and it was for this reason that the GFAAS was used as an alternate detection system. The precision was lower for the analysis of the cadmium in the GFAAS compared to the ICP-OES but due to restrictions observed with the ICP-OES, a less precise GFAAS was preferred for analysing this element. The two last metals chromium and lead were successfully analysed with high precision on the ICP-OES. The precision was high for sieved & ground random samples, highlighting the importance of proper sub-sampling techniques to improve precision. The analysis of the mercury was performed using the two detection systems; VGA-77 AAS as well as the VGA-77 ICP-OES. The precision of the VGA-77 ICP-OES system exceeded that of the ; VGA-77 AAS system as the uncertainty was lower in the analysis of the samples as well as in the recovery experiment and this was the reason the VGA-77 ICP-OES system was preferred in the analysis of the mercury.

Overall, the research conducted was able (although with some limitations) to meet all the objectives set at the beginning as operating procedures and detection systems were defined and used in the analysis of metal content in the sewage sludge. The cause and effect diagram facilitated the identification of the sources of uncertainty of which were quantified through precisions determined from the %RSD and these precisions were used in the calculation of the overall uncertainties. Recommendations for appropriate detection systems for each element were made based on the precisions obtained in the analysis.

In general, the precision of the analyses was good and in cases where the precision was poor, other alternative detection systems were used. The importance of a certified reference material was highlighted throughout and as it stands the work has no future work. However, a separate study could be done in order to verify and validate the accuracy (using a CRM) of all the analysis performed.

5. References

Aczel, A.D. & Sounderpandian, J. (2009). "Complete business statistics - Sampling methods", 7th Edition. The McGraw-Hill Companies, New York City, USA, pp. 1 – 23.

Anderson, T.M & McNaughton, J. (2006). "Nutrient acquisition and physiological responses of dominant Serengeti grasses to variation in soil texture and grazing", J Ecol. 94, pp. 1164.

Antin, L., Wu, W., Evens, A., Huang, W & Zhang, C. (2010). "Proficiency study AQA 101 metals in soil", Available at http://www.measurement.gov.au accessed: February 2013, National Measurement Institute, Australia, pp. 78 – 79, 84.

Armbruster, D.A & Pry, T (2008). "Limit of Blank, Limit of Detection and Limit of Quantitation". Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2556583/pdf/cbr29_s_pgs49.pdf accessed: January 2016, The Clinical Biochemist Reviews Vol. 29 (Suppl 1), pp. 1 – 4.

Berghof, (1996). "Theory of sample preparation using Acid digestion, Pressure digestion and Microwave digestion (Microwave decomposition)". Available at http://www.berghof.com/fileadmin/Dateien-Einpflege/Seitenbaum/Home-Downloads/Produkte/Laborgeraete/Aufschlusstechnik/MW_Theorie_Probenvorbereitung_PT_en.pdf Accessed: January 2013, Eningen, Germany, pp. 2 – 3.

Boss, C.B & Fredeen, K.J. (1997). "Concepts, Instrumentation and Techniques in Inductively Coupled Plasma Optical Emission Spectrometry", 2nd Edition, the Perkin-Elmer Corporation, USA, pp. 5 – 70.

Briggs, R., Bartram, J & Ballance, R. (1996). "Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes", Available at http://www.who.int/water_sanitation_health/resourcesquality/wqmchap9.pdf accessed: January 2016, United Nations Environment Programme and the World Health Organization, Chapter 9, pp. 1 – 17.

Chochorek, A., Bobrowski, A., Kiralyova, Z & Mocak, J. (2010). "ICP-OES Determination of Select Metals in Surface Water – a Metrological Study" Available at http://www.pjoes.com/pdf/19.1/59-64.pdf accessed: February 2012, Polish Journal of Environmental Studies, Vol. 19 (1), Poland, pp. 60 – 61.

Dean, J.R. (2003). "Methods for environmental trace analysis". Northumbria University, England. John Wiley & Sons Ltd, pp. 27 – 164.

Dominski, P. & Shrader, D.E. (2010). "Automated Cold Vapour Determination of Mercury: EPA Stannous Chloride Methodology", Available at http://www.chem.agilent.com/Library/applications/AA051.pdf accessed: July 2013, Agilent Technologies Inc., USA, pp. 1 – 8.

Duffus, J.H. (2002). "Heavy metals – a meaningless term?", Pure and Applied Chemistry, Vol. 74, no. 5. Available at http://www.szennyviztudas.bme.hu/files/iupac%20heavy%20metals.pdf, accessed: November 2012, pp. 793 – 807.

Elert, G. (1998 – 2015). "The Physics Hypertextbook", Available at http://physics.info/, accessed: April 2016, pp. 1 – 2.

Environmental Protection Agency (EPA) (1993). "Standards for use or disposal of sewage sludge", 40 CFR Parts 122, 123, 501. USA, pp. 16 – 23.

Eriksson, J. (2001). "Concentrations of 61 trace elements in sewage sludge, farmyard manure, mineral fertiliser, precipitation and in oil and crops", Available at http://swedishepa.se/Documents/publikationer/620-6246-8.pdf accessed: July 2016, Swedish Environmental Protection Agency, pp. 22 – 26.

Eshel, G., Levy, G.J., Mingelgrin, U & Singer, MJ. (2004). "Critical evaluation of the use of laser diffraction for particle-size distribution analysis". *Soil Sci. Soc. Am. J.* 68, 736 – 743.

Eurachem/CITAC guide: Measurement uncertainty arising from sampling - A guide to methods and approaches (2007), 1st edition. Available at http://www.rsc.org/images/EURACHEM1_tcm18-102815.pdf , accessed: January 2016, pp. 1 – 97.

Eurachem/CITAC guide: Quantifying uncertainty in analytical measurement (2012), 3rd edition. Available at http://www.citac.cc/QUAM2012_P1.pdf, accessed: March 2014, pp. 4 – 31.

Feldmann, J., Bralatei, E., Lacan, S & Krupp, E. M. (2015). "Detection of Inorganic Arsenic in rice using a Field Test Kit: A Screening Method", Trace Element Speciation Laboratory (TESLA), Department of Chemistry, University of Aberdeen, Scotland – United Kingdom, ACS Publications, pp. 11271 – 11276.

Gaines, P. & Brolin, B. (2003). "Reliable measurements – Ashing". Available at http://www.google.co.za/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=0ahUKEwiUrtPbmsrKAhXC2w4KHY2aBAoQFgggMAE&url=http%3A%2F%2Fimg9.chem17.com%2F5%2F20100226%2F634027781715781250.doc&usg=AFQjCNEj6n0DPosblwMtT8pfnAcZ1sN44w , accessed: January 2013, Essex Scientific Laboratory Supplies, United Kingdom, pp. 14.

Garbarino, J.R & Taylor, H.E. (1996). "Inductively Coupled Plasma – Mass Spectrometric method for the determination of dissolved trace elements in natural water", U.S Geological Survey, open – file report 94-358, Boulder Colorado, pp. 1 – 2.

GBC Scientific Equipment Pty Ltd (2005). "Optimizing Analytical Performance in ICP-OES Applications", Available at http://www.gbcscientific.com/appnotes/icp_oes_app_note_002.pdf accessed: February 2013, Australia, pp. 10 – 11.

Grzechnik, E & Pitsch, H. (2004). "The basic principles of sieve analysis". Available at http://www.ninolab.se/fileadmin/Ninolab/pdf/retsch/documents/af_sieving_basics_2004_en.pdf accessed: October 2012, Retsch GmbH & Co. KG, Germany, pp. 1 – 8.

Hibbert, D, B. (2007). "Systematic errors in analytical measurement results". Available at http://www.sciencedirect.com/science/article/pii/S002196730700492X, accessed: February 2013, Journal of Chromatography A 1158, University of New South Wales, Sydney, pp. 26.

Hope, J. (1986). "Sewage sludge disposal and utilization study". Available at http://www.wsipp.wa.gov/ReportFile/1127 , accessed: October 2012, Washington State Institute for Public Policy, Olympia: Washington, USA, pp. 3 – 11.

Huber, L. (2010). “Validation of Analytical Methods”. Available at https://www.agilent.com/cs/library/primers/Public/5990-5140EN.pdf, accessed: February 2013, Agilent Technologies, Germany, pp. 17 – 19.

Ilie, G. and Ciocoiu, C.N. (2010). “Application of fishbone diagram to determine the risk of an event with multiple causes”. Management research and practice Vol. 2 Issue. Available at http://mrp.ase.ro/no21/f1.pdf, accessed: January 2016, pp 1 – 20.

Inczédy, J, Lengyel, T & Ure, A.M. (1997). “Compendium of Analytical Nomenclature – Definitive Rules”, Available at http://old.iupac.org/publications/analytical_compendium/, accessed: February 2016, International Union of Pure & Applied Chemistry, 3rd edition, Chapter 10, pp. 1 – 2.

International Atomic Energy Agency (IAEA). (2003). “Development and use of Reference Materials and Quality Control Materials”, Available at http://www-pub.iaea.org accessed: February 2013, Austria, pp. 2 – 3.

International Organization for Standardization (ISO 11466) (1995). “Soil quality – Extraction of trace elements soluble in aqua regia”. 1st Edition, Switzerland, pp. 1 – 6.

International Organization for Standardization (ISO) 11466:1995 (E)). “Soil quality — Extraction of trace elements soluble in aqua regia”. Available for purchase at https://www.iso.org/obp/ui/#iso:std:iso:11466:ed-1:v1:en, accessed: January 2016”, pp. 1 – 12.

Jakab, G., Centeri, C., Barta, K., Bidlo, A., Szabo, S & Biro, Z. (2010). “Measuring particle size distribution: Can the differences among examined soils and methods be proven?”, Available at http://iuss.org/19th%20WCSS/Symposium/pdf/1793.pdf accessed: January 2016, 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia, pp. 90 – 93.

Kingston, H.M & Jamie, L.B. (1988). “Introduction to microwave sample preparation: Theory and practice”. ACS Professional Reference Book, ACS, Washington, pp. 263.

Lide, D. (1992). “CRC Handbook of Chemistry and Physics”, 7th Edition. Boca Raton, FL: CRC Press, pp. 1 – 57.

Lisinger, T.P.J & Josephs, D.J. (2006). “Limitations of the application of the Horwitz equation’’, Available at https://www.researchgate.net/publication/221954344_Limitations_of_the_application_of_the_Horwitz_equation, accessed: July 2016, Trends in Analytical Chemistry, *Vol.* 25 No. 11, pp. 1125.

Mdlambuzi, T. (2014). “Long-term effects of sewage sludge application on heavy metal distribution in a loam soil and indigenous vegetables’’, Available at http://researchspace.ukzn.ac.za/xmlui/bitstream/handle/10413/12119/Mdlambuzi_Thandile_2014.pdf?sequence=1 , accessed: June 2016, pp. 34 – 64.

Merck Millipore (2016). Available at http://www.merckmillipore.com/ZA/en/product/Hydrochloric-acid-32%25,MDA_CHEM-100319#anchor_COA & http://www.merckmillipore.com/ZA/en/product/Nitric-acid-65%25,MDA_CHEM-100456#anchor_COA , accessed: May 2016, pp. 1.

Milieu Ltd, RPA for the European Commission & DG Environment, (2008). “Environmental, economic and social impacts of the use of sewage sludge on land”. Available at http://ec.europa.eu/environment/archives/waste/sludge/pdf/part_ii_report.pdf , accessed: October 2012, Belgium, pp. 1 – 15.

Mitra, S. & Brukh, R. (2003). “Sample preparation: An analytical perspective; Sample Preparation Techniques in Analytical Chemistry”. Available at http://cnqzu.com/library/Anarchy%20Folder/Chemistry/MISC/Sample%20Preparation%20Techniques%20in%20Analytical%20Chemistry%20(Wiley,%202003).pdf, accessed: October 2012, Newark, New Jersey, John Wiley & Sons, Inc.**:** 36., pp. 1 – 2.

Moffat, A. (2006). “Use of sewage sludges and composts in Forestry”, Environmental and Human Sciences Division, Available at http://www.forestry.gov.uk/pdf/fcin079.pdf/$FILE/fcin079.pdf, accessed: September 2012, Forest Research, Edinburgh: Scotland, pp. 2 – 5.

Molenberghs, G. (2004). “Survey methods and Sampling techniques”. Interuniversity Institute for Biostatistics and Statistical Bioinformatics. Belgium, Hasselt University, Master in Statistics and Quantitative Methods 882.

PerkinElmer Inc., (2004). “AAnalyst 600/800 Atomic Absorption Spectrometers”, Available at http://www.tajhizshimi.com/BRO_AAnalyst600800[1].pdf accessed: April 2013, USA, pp. 1 – 11.

PerkinElmer, Inc. (2009). “Atomic Spectroscopy: A guide to selecting the appropriate technique and system”. Available at https://www.perkinelmer.com/CMSResources/Images/44-74482BRO_WorldLeaderAAICPMSICPMS.pdf , accessed: November 2012, Waltham, USA, pp. 1 - 15. Saskatchewan Environment, Canada, pp. 1 – 5.

Shamuyarira, K.K & Gumbo, J.R. (2014). “Assessment of Heavy Metals in Municipal Sewage Sludge: A Case Study of Limpopo Province, South Africa”, Available at http://www.mdpi.com/1660-4601/11/3/2569, accessed: July 2014, *International Journal of Environmental Research and Public Health*, pp. 2574 – 2575.

Sterritt, R.M & Lester, J.N. (1980). “Concentrations of heavy metals in forty sewage sludges in England”, Available at http://link.springer.com/article/10.1007%2FBF00291831, accessed: July 2016, Public Health Engineering Laboratory, Department of Civil Engineering, Imperial College of Science and Technology, London SW7, England, pp. 129.

The Cooperation on International Traceability in Analytical Chemistry (CITAC) & Eurachem (2002). “Guide to Quality in Analytical Chemistry”. Available at http://www.citac.cc/CITAC_EURACHEM_GUIDE.pdf, accessed: February 2013, CITAC & EURACHEM Publishers, 33 – 34.

Thermoscientific (2011). “Applications Tip of the Week: Matrix Spiking – Why Spike and How to Do It”, Available at https://static.fishersci.com/cmsassets/downloads/segment/Scientific/pdf/WaterAnalysis/Log112tipMatrixSpikeWhySpikeHowtoDoIt.pdf , accessed: May 2016, Environmental & Process Instruments Division (EPD) & Water Analysis Instruments (WAI), pp. 1 – 2.

Thompson, M., Ellison, SR., Fajgelj, A., Willetts, P & Wood, R. (1999). “Harmonized guidelines for the use of recovery information in analytical measurement (Technical Report)”, Available at http://lagrored.marm.es/DocsPublicos/Guidelines%20recovery%20IUPAC.pdf accessed: January 2016, international union of pure and applied chemistry, Vol. 71, No. 2, pp. 337 – 348.

Thompson, M & Walsh, J.N. (1983). “A handbook of inductively coupled plasma spectrometry”. 1st edition, Glasgow: Blackie & Son.

Tiruneh, A.T, Fadiran, A.O & Mtshali, J.S. (2014). “Evaluation of the risk of heavy metals in sewage sludge intended for agricultural application in Swaziland”, Available at http://www.ipublishing.co.in/ijesarticles/fourteen/articles/volfive/EIJES51017.pdf, accessed: July 2016, *International Journal of Environmental Sciences, Vol* 5 No. 1, pp. 205 – 206.

United Nations Office on Drugs and Crime (UNODC). (2009). “Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens”. Available at https://www.unodc.org/documents/scientific/validation_E.pdf, accessed: February 2013, New York, pp. 11 – 12.

Varian Inc., (2004). “VGA-77 Operation Manual”, Available at http://www.agilent.com/cs/library/usermanuals/public/1047.pdf accessed: February 2013, Varian Australia Pty Ltd, pp. 1 – 69.

Wiechmann, B., Dienemann, C., Kabbe, C., Brandt, S., Vogel, I., Roskoch, A. (2011). “Sewage sludge management in Germany”. Available at https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/sewage_sludge_management_in_germany.pdf accessed: January 2016, Umweltbundesamt (UBA) Publishers Postfach 1406, pp. 1 – 10.

Appendix

A: *Calculations for the determinatin of moisture content & final concentrations in mg. kg^{-1}*

1. The calculation of the moisture content of the sewage sludge:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight on drying (g)}}{\text{Initial sample mass (g)}} \text{ x } 100$$

$$\text{Loss in weight (g)} = \text{Initial sample mass (g)} - \text{Final mass after drying (g)}$$

2. The calculation of the concentration in mg.kg $^{-1}$:

$$\text{Concentration (mg. kg}^{-1}) = \frac{\text{Concentration } \left(\frac{\text{mg}}{\text{L}}\right) \text{x (100 ml/1000)}}{\text{Sample mass (kg)}}$$

- where Concentration $\left(\frac{\text{mg}}{\text{L}}\right)$ is a concentration obtained from the ICP-OES

3. The calculation of the cadmium concentration in mg.kg $^{-1}$:

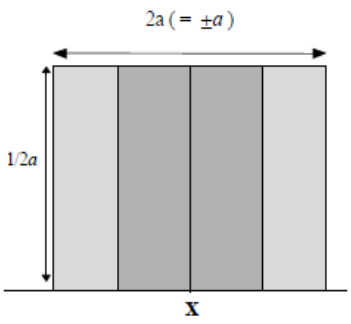
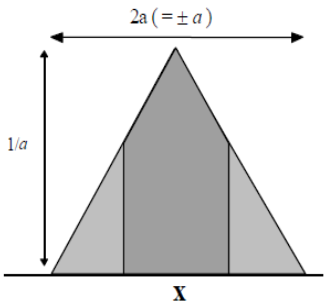
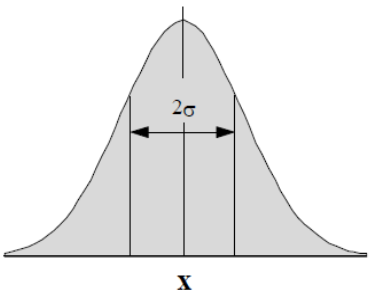
$$\text{Concentration (mg. kg}^{-1}) = \frac{\text{Concentration } \left(\frac{\mu\text{g}}{\text{L}}\right) \text{x } \left(100 \text{ x } \frac{1\text{ml}}{1000}\right) \text{ x (1 mg/1000}\mu\text{g)}}{\text{Sample mass (kg)}}$$

- where Concentration $\left(\frac{\mu\text{g}}{\text{L}}\right)$ is a concentration obtained from the GFAAS

B: ***The T-test: Two-Sample Assuming Unequal Variances for comparison of the mean concentrations of the analytes obtained from coned & quartered samples against random samples***

	Variable 1	*Variable 2*	
Mean	20.175	20.205	
Variance	54.3139	56.28923333	
Observations	4	4	
Hypothesized Mean Difference	0		
df	6		
t Stat	-0.005705156		
P(T<=t) one-tail	0.497816464		
t Critical one-tail	1.943180281		
P(T<=t) two-tail	0.995632927	> 0.05 therefore not significantly different.	
t Critical two-tail	2.446911851		

C: *Distribution Functions* (Eurachem, 2012)

<table>
<tr><th colspan="3">Rectangular Distribution</th></tr>
<tr><td>Form</td><td>Use when:</td><td>Uncertainty</td></tr>
<tr><td> 
</td><td>• A certificate or other specification gives limits without specifying a level of confidence (e.g. 25 mL ± 0.05 mL)
• An estimate is made in the form of a maximum range (±a) with no knowledge of the shape of the distribution.</td><td>$u(x) = \frac{a}{\sqrt{3}}$</td></tr>
<tr><th colspan="3">Triangular Distribution</th></tr>
<tr><td>Form</td><td>Use when:</td><td>Uncertainty</td></tr>
<tr><td> 
</td><td>• The available information concerning x is less limited than for a rectangular distribution. Values close to x are more likely than near the bounds.
• An estimate is made in the form of a maximum range (±a) described by a systematic distribution.</td><td>$u(x) = \frac{a}{\sqrt{6}}$</td></tr>
<tr><th colspan="3">Normal Distribution</th></tr>
<tr><td>Form</td><td>Use when:</td><td>Uncertainty</td></tr>
<tr><td> 
</td><td>• An estimate is made from repeated observations of a randomly varying process.
• An uncertainty is given in the form of a standard deviation$s$, a relative standard deviation $s/\bar{x}$, or percentage coefficient of variance %CV without specifying the distribution.</td><td>$u(x) = \mathrm{s}$

$u(x) = \mathrm{s}$
$u(x) = x\,.\,(s/\bar{x}\,)$
$u(x) = \left(\%\frac{CV}{100}\right).x$</td></tr>
</table>

	• An uncertainty is given in the form of a 95% (or other) confidence interval without specifying the distribution $x \pm c$.	$u(x) = \frac{c}{2}\ (for\ c\ at\ 95\%)$ $u(x) = \frac{c}{3}\ (for\ c\ at\ 99.7\%)$